Monolith X Protocol MOX-P-111

DNA Aptamer – AMP - Thermodynamics

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 temperature dependency of the DNA aptamer – AMP affinity can be used to determine enthalpic (ΔH) and entropic (ΔS) contributions of the molecular interaction via a Van't Hoff analysis.

nucleic acid – small molecule | DNA | aptamer | thermodynamics

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A1. Target/Fluorescent Molecule

DNA aptamer for adenosine

A2. Molecule Class/Organism

DNA aptamer

A3. Sequence/Formula

5' Cy5 ACC TGG GGG AGT ATT GCG GAG GAA GGT 3'

A4. Purification Strategy/Source

metabion international AG

A5. Stock Concentration/Stock Buffer

0.90 mg/ml | 100 µM
ddH_2O

A6. Molecular Weight/Extinction Coefficient

9019 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[1]

A8. Labeling Strategy

Cy5

[1] Reaction buffer C from Control Kit RED (MO-C030, NanoTemper Technologies GmbH)

A9. Labeling Procedure

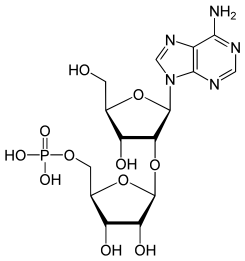
1. Mix 2 µL of 100 µM aptamer with 5 mL of dilution buffer to obtain a 40 nM aptamer solution.
2. Prepare 200 µL aliquots and store at -20°C.

A10. Labeling Efficiency

HPLC-purified, 100% labeled oligonucleotide

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

B6. Molecular Weight/Extinction Coefficient

347.22 Da

B7. Serial Dilution Preparation

1. Prepare a PCR-rack with 16 PCR tubes. Mix 4 µL of the 50 mM AMP solution with 16 µL of dilution buffer in 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 40 nM AMP aptamer to each tube from **16** to **1** and mix by pipetting.
4. Load capillaries immediately.

D1. Monolith System/Capillaries

Monolith X (NanoTemper Technologies GmbH)
Monolith Premium Capillaries (MO-K025, NanoTemper Technologies GmbH)

D2. Monolith Software

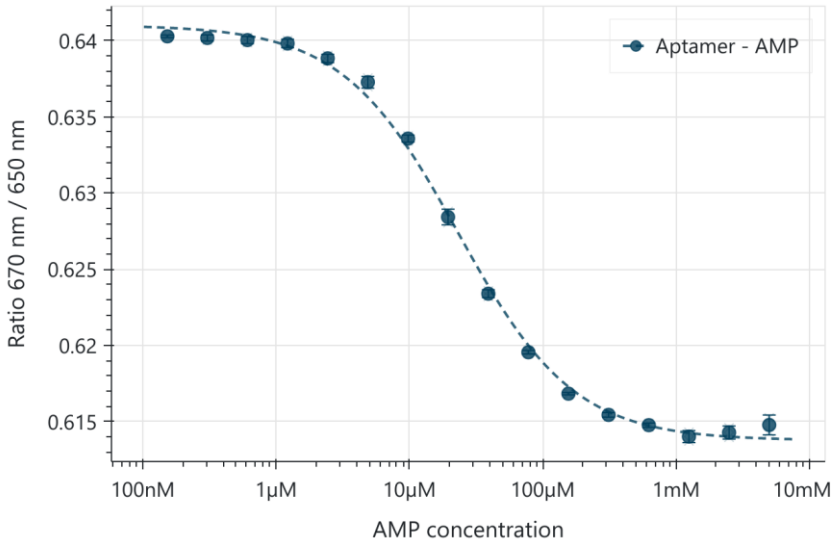
MO.Control v2.4.2 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

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

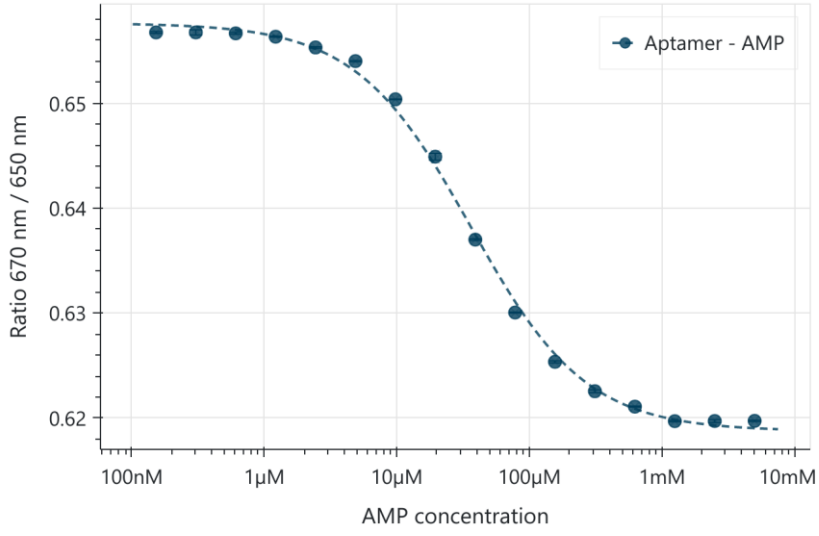
20 mM Tris, pH 7.8, 300 mM NaCl, 5 mM $MgCl_2$, 0.05% TWEEN® 20
20 nM aptamer | 5 mM – 153 nM AMP | 20°C – 40°C | 40% excitation power

D4. Monolith Results (Dose Response)

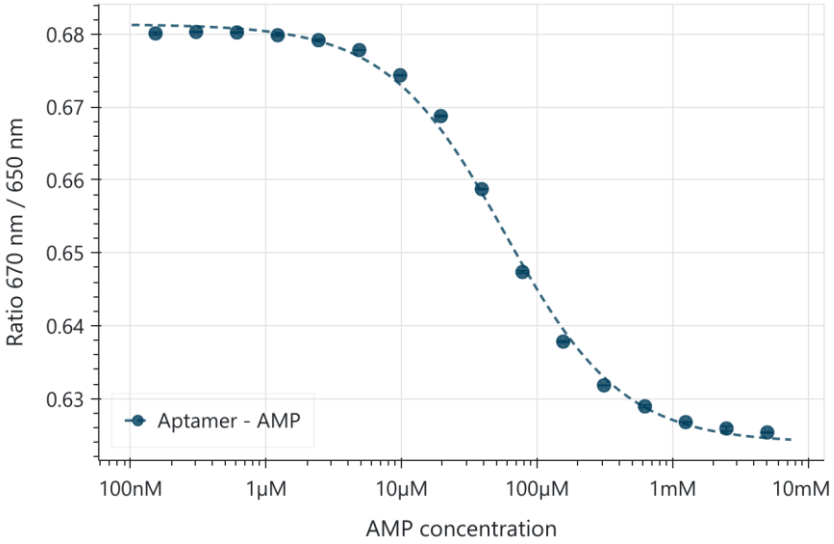
20°C | K_d = 23.8 ± 1.4 µM (S/N = 53.1)



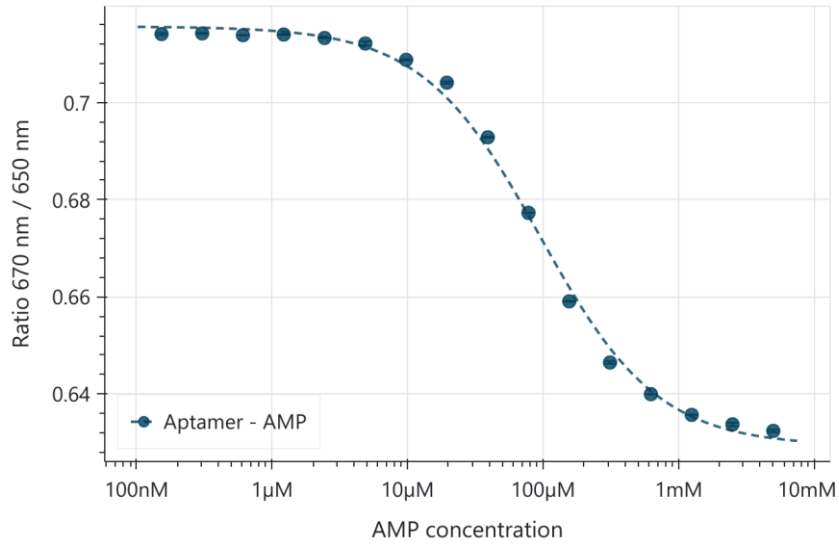
25°C | K_d = 37.3 ± 2 µM (S/N = 59.6)



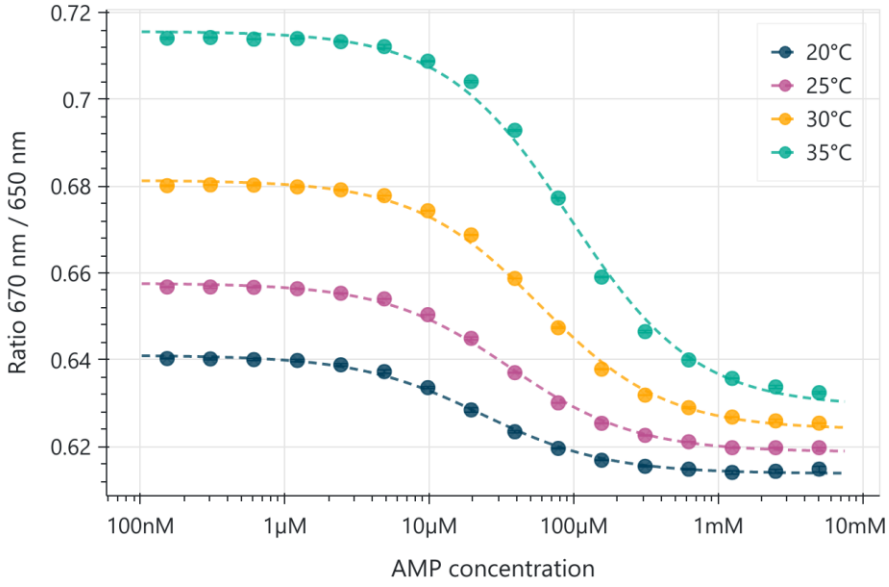
30°C | K_d = 59.7 ± 3.3 µM (S/N = 57.5)



35°C | K_d = 98.1 ± 6.5 µM (S/N = 48.9)



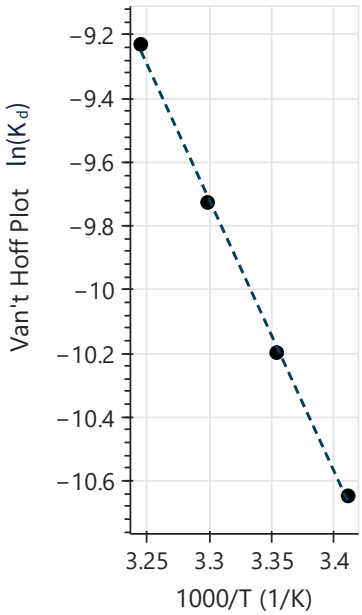
Overlay:

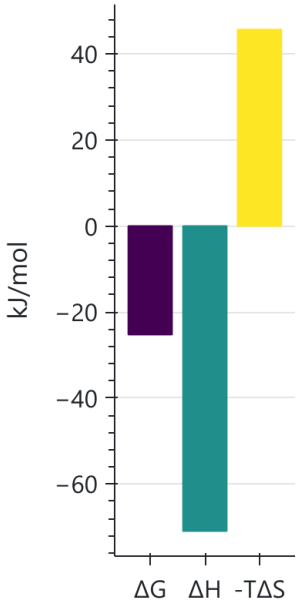


Overview of determined K_d values at different temperatures:

T (°C)	20	25	30	35
K_d (µM)	23.8	37.3	59.7	89.1

Van't Hoff analysis[2]:





ΔH = −70.9 ± 1.9 kJ/mol
ΔS = −153.2 ± 6.2 J/mol/K
ΔG (at 25°C) = −25.2 ± 2.6 kJ/mol

[2]Calculations can be performed with Monolith X's Thermodyanmics Measurement mode in MO.Control 2.7.0 and later versions. Plots were created outside of MO.Control 2 with temperature set at 25°C (298.15K) for ΔG calculation.

D5. Reference Results/Supporting Results

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

E. Contributors

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