Monolith Protocol MO-P-023

Thermodynamics of DNA Hybridization

The stability of DNA duplexes depends on a fine balance of hydrogen bonds and base-stacking interactions. Factors affecting stability are ionic strength and temperature. Monitoring the shift in the dissociation constant (K_D) of the hybridization interaction over a range of temperatures can be used to calculate the free enthalpy (ΔH) and entropy (ΔS) for the hybridization reaction.

DNA – DNA interaction | Van't Hoff analysis | ΔH | ΔS

A1. Target/Fluorescent Molecule

GB1

A2. Molecule Class/Organism

DNA

A3. Sequence/Formula

5' Cy5 GGA CTT CAG G 3'

A4. Purification Strategy/Source

metabion international AG

A5. Stock Concentration/Stock Buffer

100 µM
ddH_2O

A6. Molecular Weight/Extinction Coefficient

3602 Da
98,000 $M^{-1}cm^{-1}$ (ε_{260})

A7. Dilution Buffer

100 mM NaCl, 2 mM KCl, 7.2 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.4, 0.1% TWEEN® 20

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

aGB1

B2. Molecule Class/Organism

DNA

B3. Sequence/Formula

5’ CCT GAA GTC C 3’

B4. Purification Strategy/Source

metabion international AG

B5. Stock Concentration/Stock Buffer

200 µM
ddH_2O

B6. Molecular Weight/Extinction Coefficient

2988 Da
91,200 $M^{-1}cm^{-1}$ (ε_{260})

B7. Serial Dilution Preparation

1. Prepare a PCR-rack with 16 PCR tubes. Transfer 10 µL of ddH_2O[1] into tubes **1** to **16**.
2. Add 10 µL of 200 µM aGB1 to tube **1** and mix by pipetting. Then, 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. Mix 2 µL of 100 µM GB1 with 48 µL of ddH_2O to obtain 50 µL of a 4 µM GB1 solution.
4. Mix 2 µL of 4 µM GB1 with 198 µL of dilution buffer to obtain 200 µL of a 40 nM GB1 solution.
5. Mix 100 µL of 40 nM GB1 with 100 µL of dilution buffer to obtain 200 µL of a 20 nM GB1 solution.
6. Add 10 µL of 20 nM GB1 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.

[1] Pipetting detergent-free H_2O reduces pipetting errors which is important for later analysis of the initial fluorescence.

D1. MST System/Capillaries

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

D2. MST Software

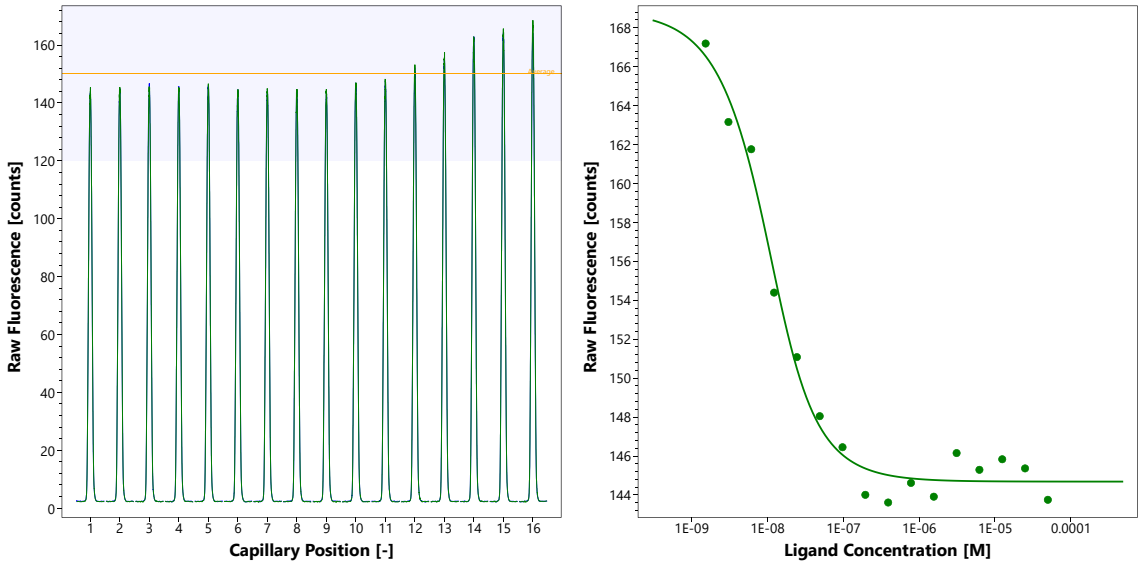
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

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

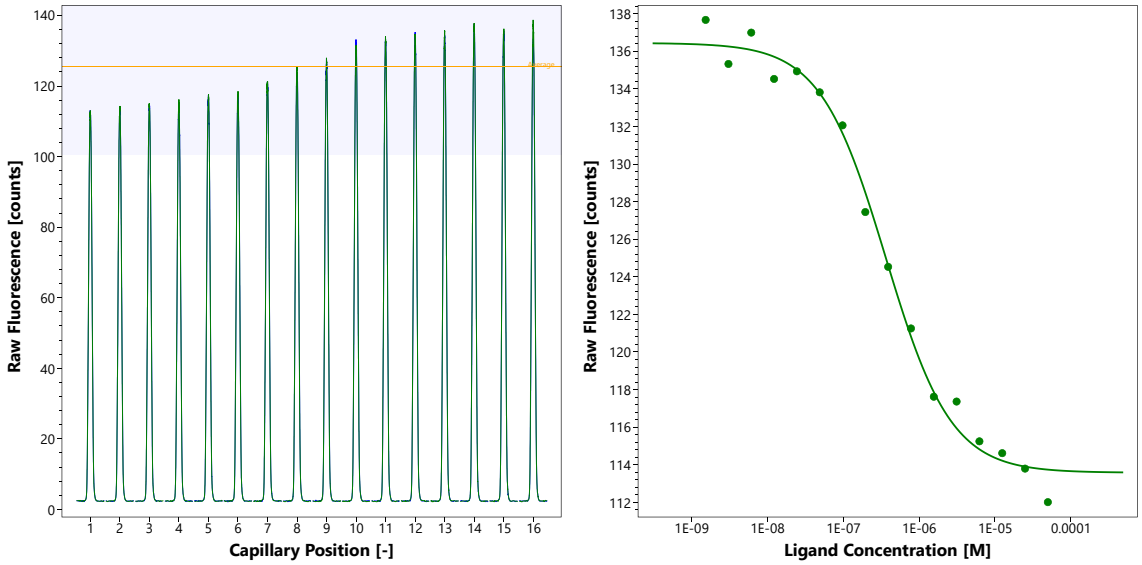
50 mM NaCl, 1 mM KCl, 3.6 mM Na_2HPO_4, 0.7 mM KH_2PO_4, pH 7.4, 0.05% TWEEN® 20
10 nM GB1 | 50 µM – 1.52 nM aGB1 | 22°C – 32°C | low MST power | 20% excitation power

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

K_d = 5.32 nM (22°C) Initial Fluorescence Analysis



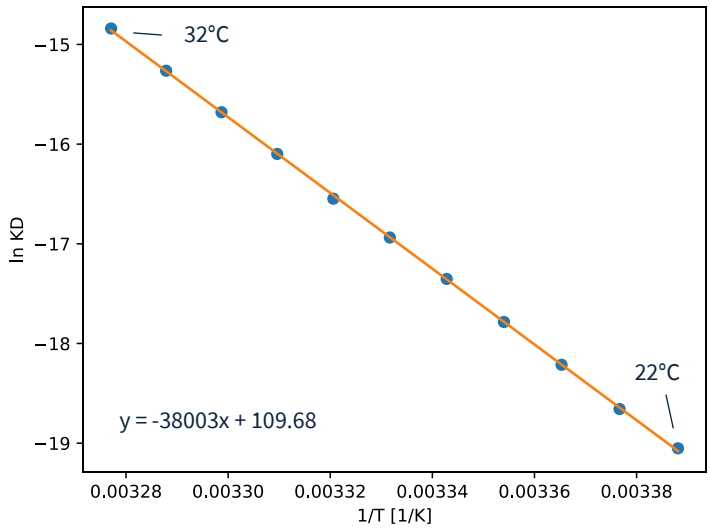
K_d = 359 nM (32°C) Initial Fluorescence Analysis



Overview of determined K_d values from Initial Fluorescence Analysis at different temperatures:

T (°C)	22	23	24	25	26	27	28	29	30	31	32
K_D (nM)	5.32	7.89	12.3	18.9	29.1	44.1	65.1	102	155	235	359

Van't Hoff plot:



$$\Delta H = \text{slope} \cdot R = -38003\,K \cdot 8.314 \frac{J}{mol \cdot K} = -315.96 \frac{kJ}{mol} = -75.51 \frac{kcal}{mol}$$

$$\Delta S = -\text{intercept} \cdot R = -109.68 \cdot 8.314 \frac{J}{mol \cdot K} = -911.88 \frac{J}{mol \cdot K} = -217.94 \frac{cal}{mol \cdot K}$$

D5. Reference Results/Supporting Results

K_d = 11.6 nM (23°C) sequence CTCACAACAG	Surface Plasmon Resonance (SPR) W Palau and C Di Primo, Biochimie 94 (2012) 1891-1899
K_d = 6.77 nM (23°C) sequence CTCACAACAG	switchSENSE Dynamic Biosensors, Application Note
ΔH = -72.6 kcal/mol ΔS = -208.2 cal/(mol K)	DNA Thermodynamics Calculator biophysics.idtdna.com

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