Monolith X Protocol MOX-P-115

DNA Hybridization - Thermodynamics

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.

nucleic acid – nucleic acid | DNA | hybridization | thermodynamics | van't Hoff analysis | enthalpy | entropy

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A1. Target/Fluorescent Molecule

Cy5-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

0.36 mg/ml | 100 µM
ddH_2O

A6. Molecular Weight/Extinction Coefficient

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

A7. Dilution Buffer

40 mM sodium phosphate buffer, pH 7.2, 200 mM NaCl, 0.1% TWEEN® 20[1]

A8. Labeling Strategy

Cy5

[1] 2X assay buffer

A9. Labeling Procedure

1. Prepare the dilution buffer by dissolving 236 mg of Na_2HPO_4 (MW: 177.99 g/mol), 105 mg of NaH_2PO_4 (MW: 156.01 g/mol) and 584 mg of NaCl (MW: 58.44 g/mol) in 49.5 mL of ddH_2O. Then, add 500 µL of 10% TWEEN® 20.
2. Dilute 1.5 µL of 100 µM Cy5-GB1 in 15 mL of dilution buffer to obtain a 10 nM Cy5-GB1 solution.
3. Prepare 180 µL aliquots and store at -80°C.

A10. Labeling Efficiency

HPLC-purified, 100% labeled oligonucleotide

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

0.60 mg/ml | 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 180 µL of 10 nM Cy5-GB1.
2. Prepare a PCR-rack with 16 PCR tubes. Mix 2 µL of 200 µM aGB1 with 18 µL of ddH_2O in tube **1**. Then, transfer 10 µL of ddH_2O 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 10 nM Cy5-GB1 to each tube from **16** to **1** and mix by pipetting.
5. Load capillaries[2].
6. Set the Monolith capillary tray temperature to the desired value and wait at least 5 minutes before starting the measurement to ensure sufficient equilibration of the system.

[2] When performing multiple measurements at different temperatures, seal capillaries using the Capillary Sealing Paste Prometheus Series (PR-P001, NanoTemper Technologies GmbH).

D1. Monolith System/Capillaries

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

D2. Monolith Software

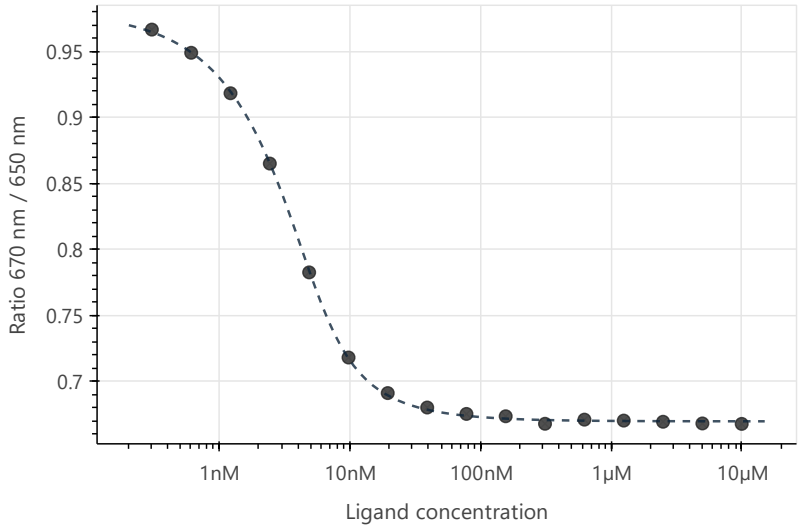
MO.Control v2.4.2 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

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

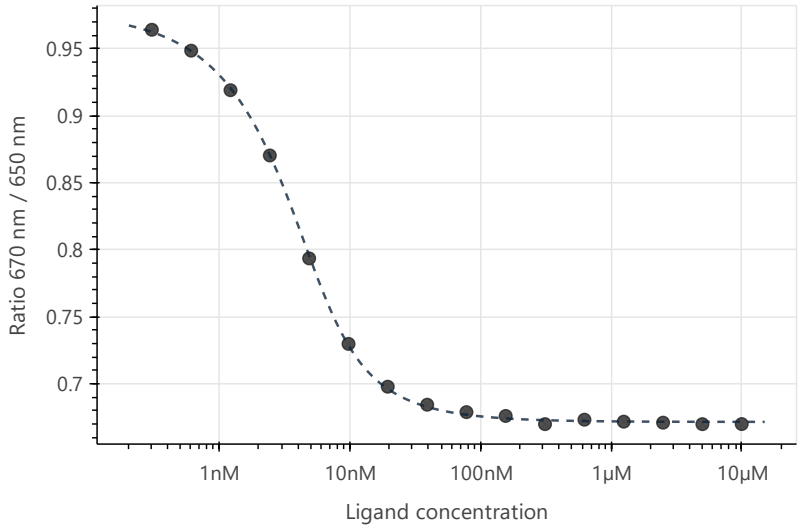
20 mM sodium phosphate buffer, pH 7.2, 100 mM NaCl, 0.05% TWEEN® 20
5 nM Cy5-GB1 | 10 µM – 305 pM aGB1 | 20°C – 37°C | 100% excitation power

D4. Monolith Results (Dose Response)

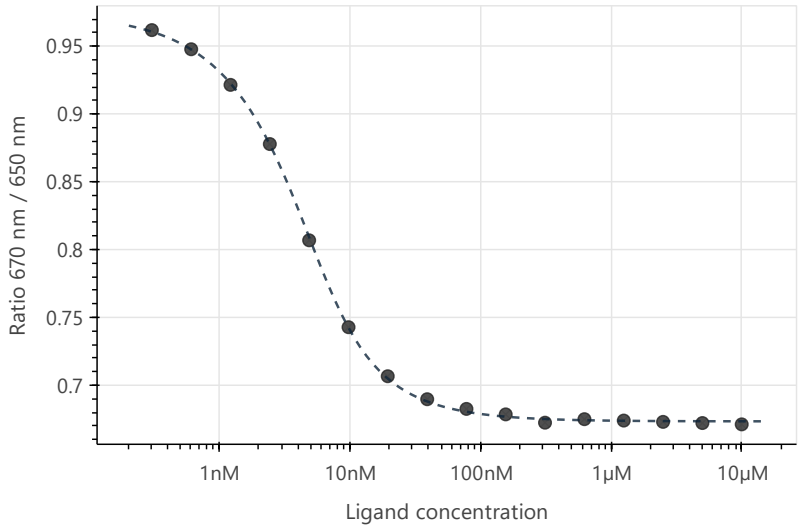
20°C | K_d = 1.00 ± 0.03 nM(S/N = 203.9)



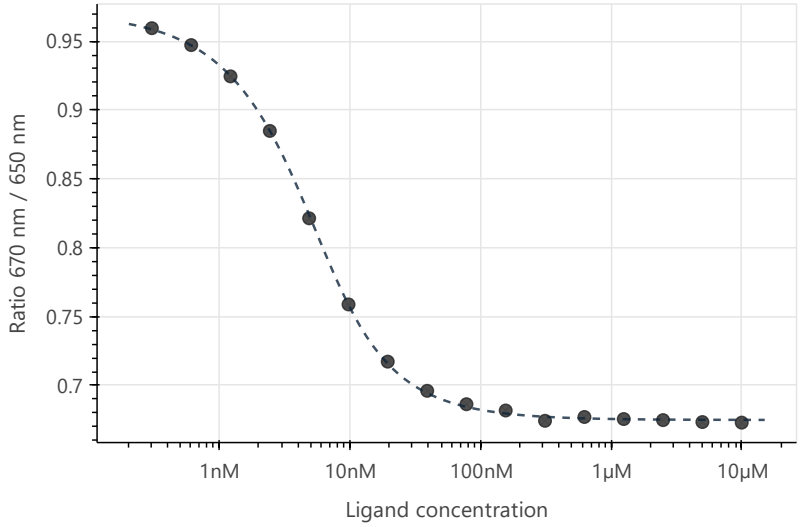
21°C | K_d = 1.31 nM ± 0.04 (S/N = 187.3)



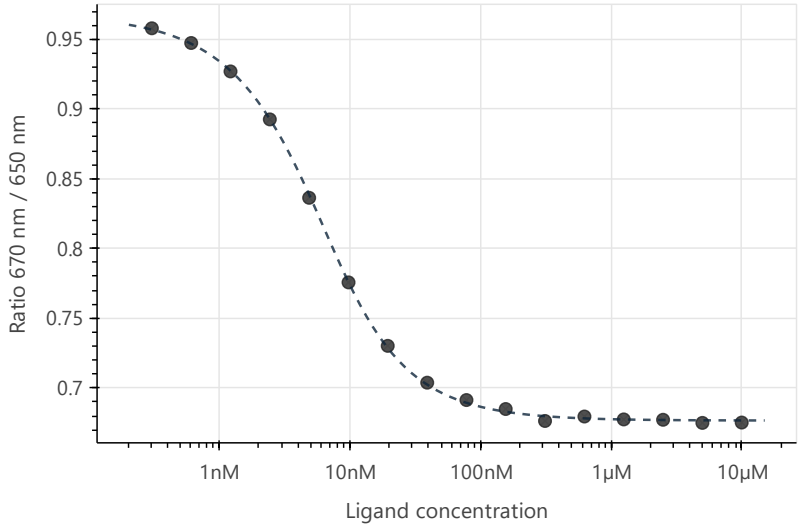
22°C | K_d = 1.76 ± 0.05 nM (S/N = 192.4)



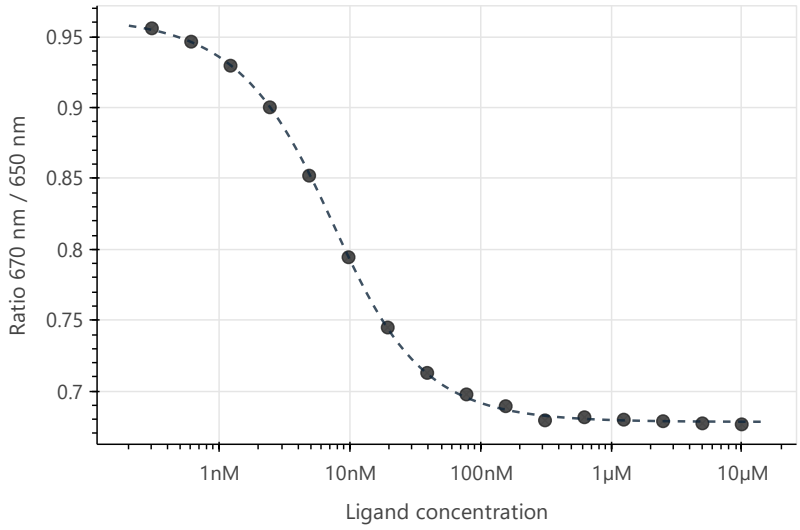
23°C | K_d = 2.44 ± 0.06 nM (S/N = 187.8)



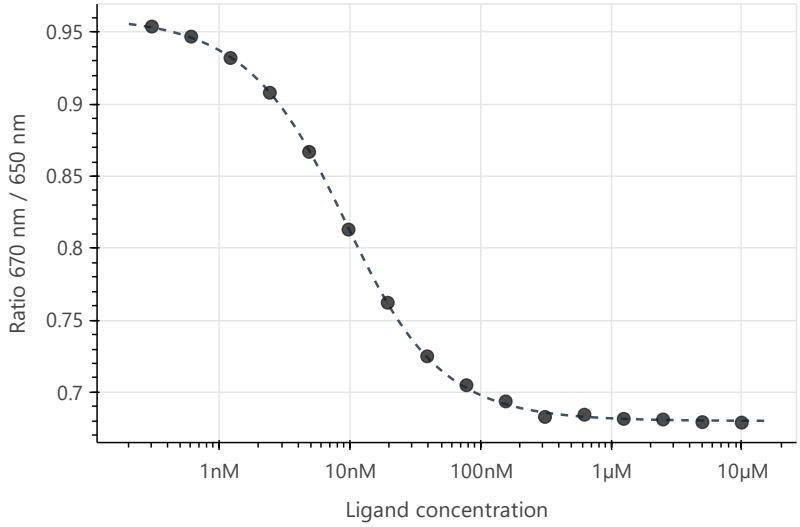
24°C | K_d = 3.35 ± 0.08 nM (S/N = 181.6)



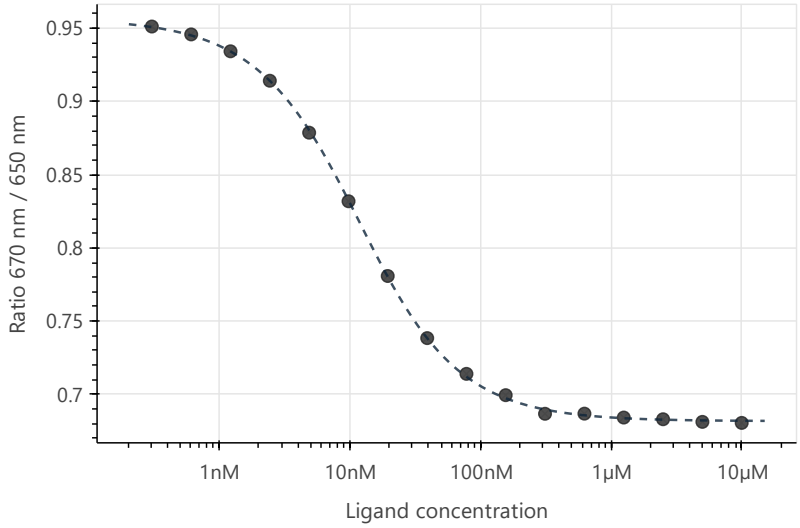
25°C | K_d = 4.69 nM ± 0.1 (S/N = 192.0)



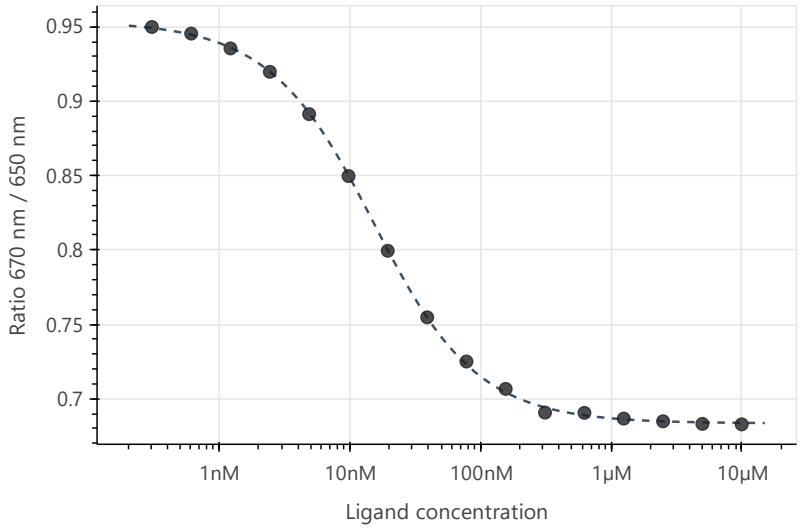
26°C | K_d = 6.53 nM ± 0.12 (S/N = 213.2)



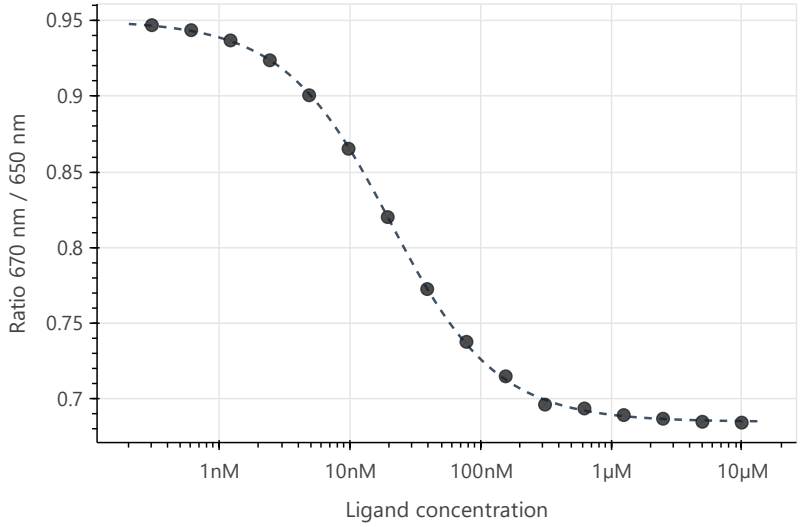
27°C | K_d = 9.11 nM ± 0.16 (S/N = 208.8)



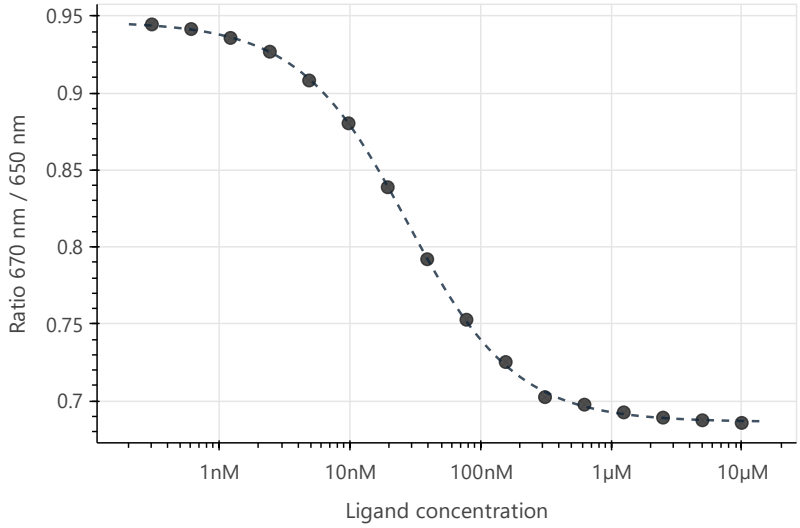
28°C | K_d = 12.6 ± 0.2 nM (S/N = 193.2)



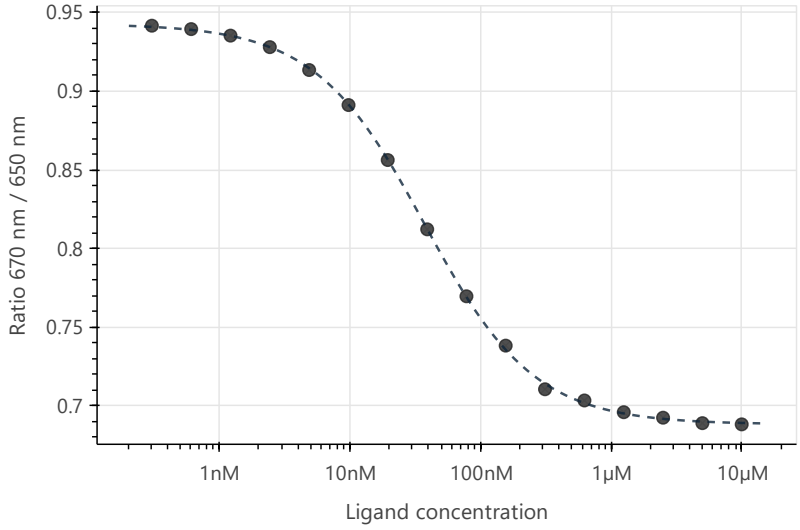
29°C | K_d = 17.7 ± 0.3 nM (S/N = 207.1)



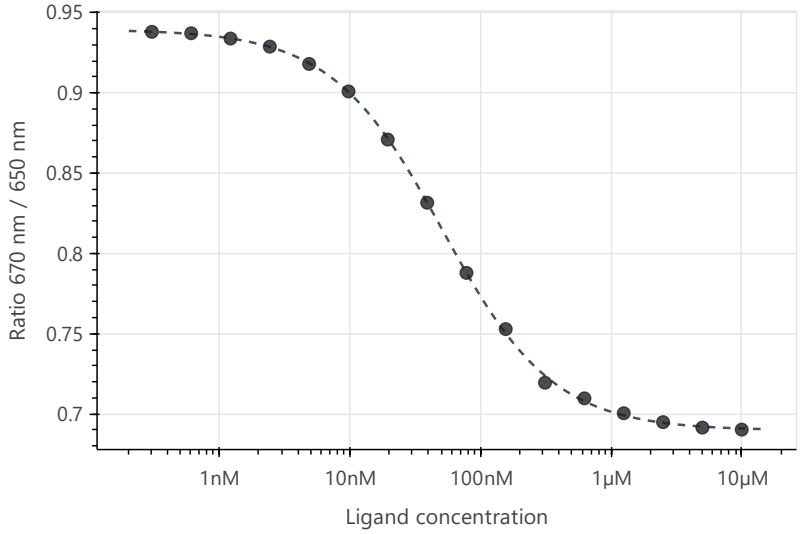
30°C | K_d = 24.9 ± 0.4 nM (S/N = 204.1)



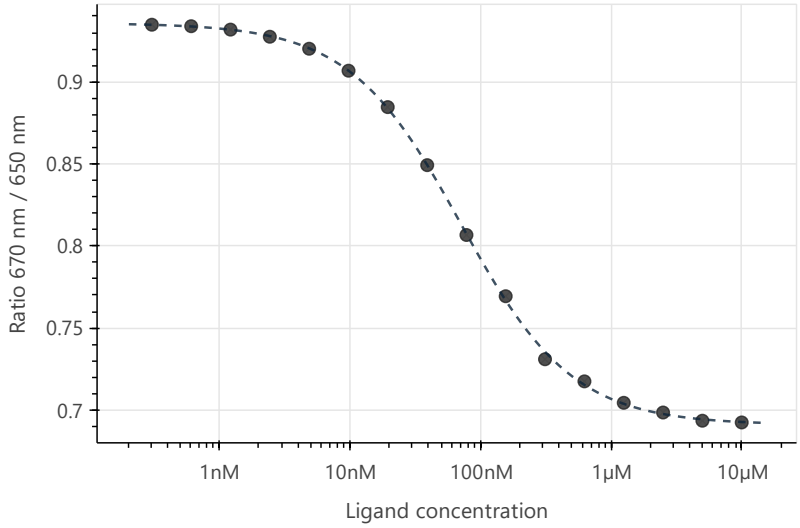
31°C | K_d = 34.8 ± 0.6 nM (S/N = 185.5)



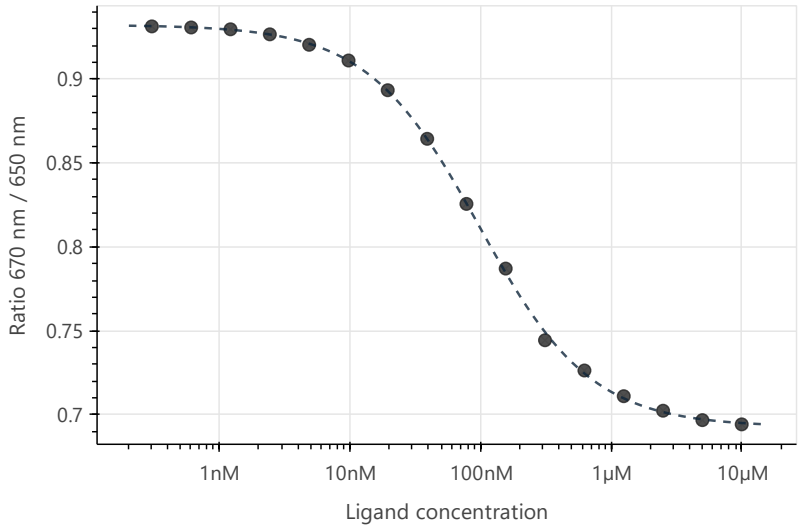
32°C | K_d = 48.8 ± 1 nM (S/N = 166.5)



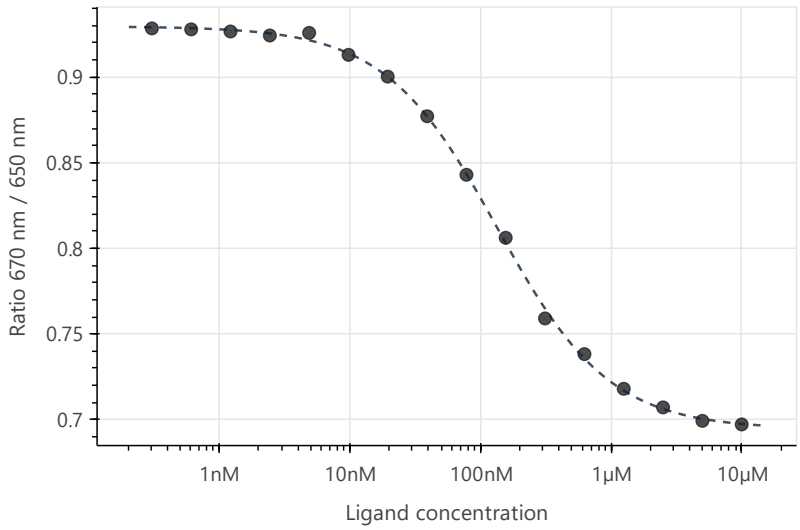
33°C | K_d = 68.9 ± 1.4 nM (S/N = 158.1)



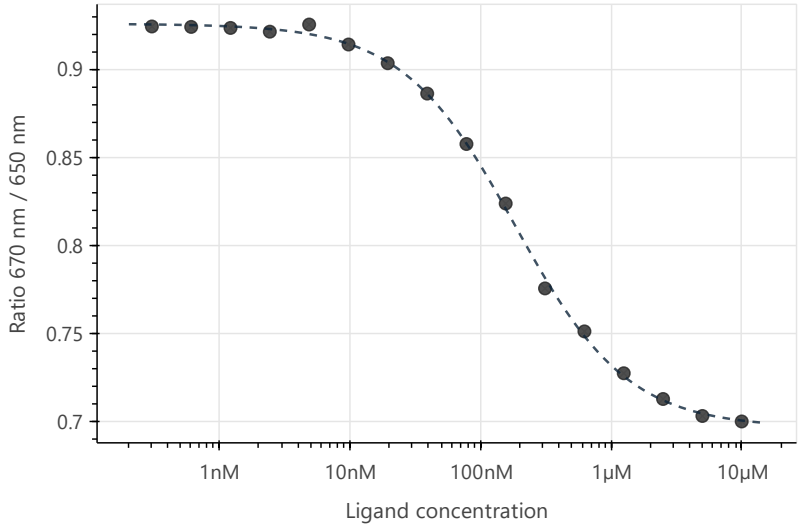
34°C | K_d = 96.6 ± 2 nM (S/N = 151.1)



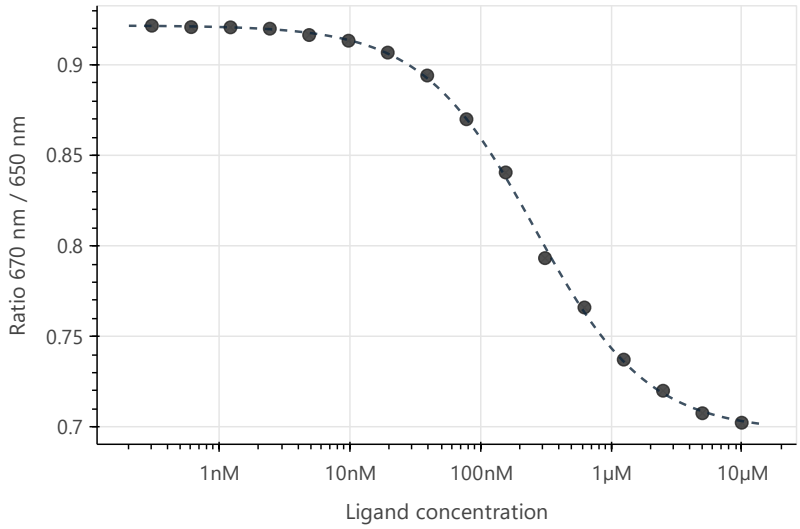
35°C | K_d = 134 ± 4 nM (S/N = 103.4)



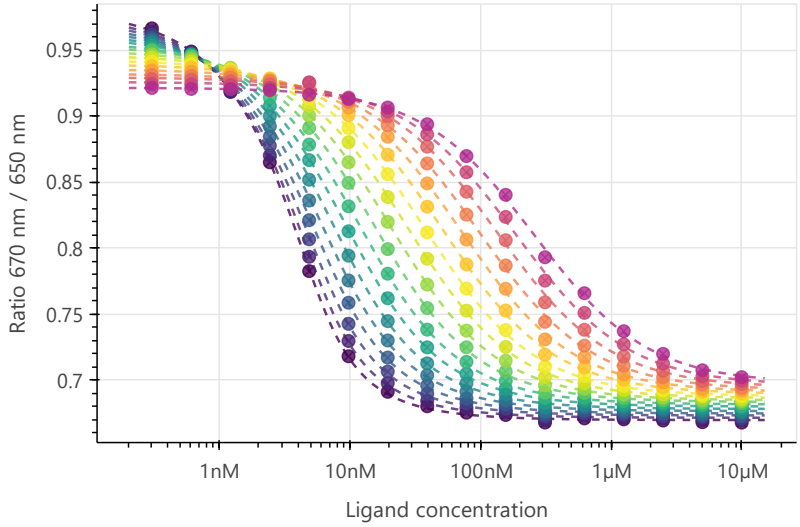
36°C | K_d = 182 ± 7 nM (S/N = 90.7)



37°C | K_d = 257 ± 8 nM (S/N = 106.7)



Overlay

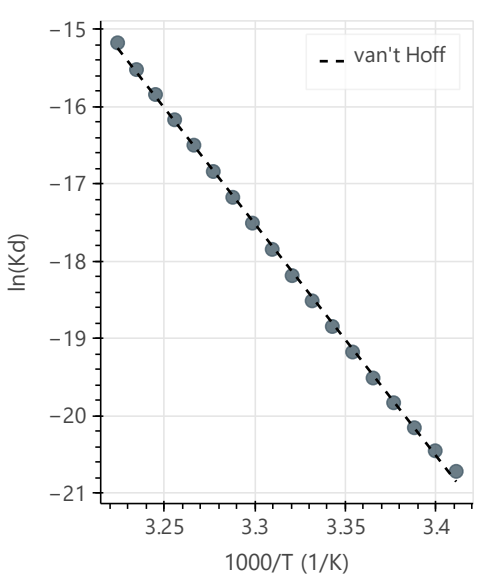


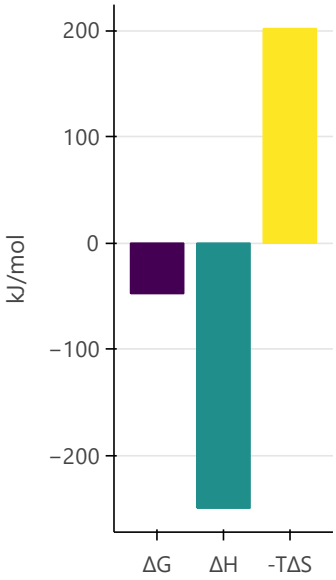
Overview of determined K_d values at different temperatures:

T (°C)	20	21	22	23	24	25	26	27	28
K_d (nM)	1.00	1.31	1.76	2.44	3.35	4.69	6.52	9.11	12.6

T (°C)	29	30	31	32	33	34	35	36	37
K_d (nM)	17.7	24.9	34.8	48.8	68.9	96.6	134	186	257

Van't Hoff analysis[3]:





$$\Delta H = \text{slope} \cdot R = -30096\ \text{K} \cdot 8.314 \frac{\text{J}}{\text{mol} \cdot \text{K}} = -250.2 \frac{\text{kJ}}{\text{mol}} = -59.8 \frac{\text{kcal}}{\text{mol}}$$

$$\Delta S = -\text{intercept} \cdot R = -81.804 \cdot 8.314 \frac{\text{J}}{\text{mol} \cdot \text{K}} = -680.1 \frac{\text{J}}{\text{mol} \cdot \text{K}} = -162.4 \frac{\text{cal}}{\text{mol} \cdot \text{K}}$$

D5. Reference Results/Supporting Results

K_d = 11.6 nM (23°C) sequence CTC ACA ACA G	Surface Plasmon Resonance (SPR) Palau and Di Primo, Biochimie 94 (2012) 1891-1899
K_d = 6.77 nM (23°C) sequence CTC ACA ACA G	switchSENSE Dynamic Biosensors, Application Note
K_d = 3.9 nM (25°C) | ΔH = –56.1 kcal/mol | ΔS = –149.5 cal/(mol K) sequence GAC GTG CGA AG	Isothermal Titration Calorimetry (ITC) Halvorsen et al., Anal Biochem 465 (2014) 127–133

E. Contributors

Andreas Langer[4]

[3] Calculations can be performed with Monolith X's Thermodynamics 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.
[4] NanoTemper Technologies GmbH, München, Germany | nanotempertech.com