Monolith X Protocol MOX-P-108

BoxP1 Hairpin DNA – Mlc

Mlc (*makes large colonies*) from *Escherichia coli* is a transcriptional repressor which controls the expression of several genes encoding enzymes involved in glucose metabolism. Mlc forms stable homodimers in order to bind DNA, though it usually is found as a tetramer in studies using size exclusion chromatography. The hairpin DNA is a model for the operator sequence that Mlc interacts with. This interaction has been characterized with SPR and ITC as well.

protein – nucleic acid | gene regulation

A1. Target/Fluorescent Molecule

BoxP1 Hairpin

A2. Molecule Class/Organism

Double-stranded DNA (Hairpin)
Escherichia coli

A3. Sequence/Formula

5’ Cy5 TTA TTT TAC TCT GTG TAA TAA ATC CCC AAA AAT TTA TTA CAC AGA GTA AAA TAA 3’

A4. Purification Strategy/Source

metabion international AG

A5. Stock Concentration/Stock Buffer

300 µM
ddH_2O

A6. Molecular Weight/Extinction Coefficient

17.1kDa
559,000 $M^{-1}cm^{-1}$ (ε_{260})

A7. Dilution Buffer

10 mM HEPES, pH 7.2, 150 mM NaCl, 10 mM KCl, 1 mM $MgCl_2$, 0.5 mM EDTA

A8. Labeling Strategy

Cy5

A9. Labeling Procedure

1. Mix 2 µL of 300 µM BoxP1 Hairpin with 598 µL of dilution buffer to obtain 600 µL of a 1 µM BoxP1 Hairpin solution.
2. Prepare 10 µL aliquots and store at -80°C.

A10. Labeling Efficiency

HPLC-purified, 100% labeled oligonucleotide

B1. Ligand/Non-Fluorescent Binding Partner

Mlc
uniprot.org/uniprot/P50456

B2. Molecule Class/Organism

Transcriptional repressor protein
Escherichia coli

B3. Sequence/Formula

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MVAENQPGHI DQIKQTNAGA VYRLIDQLGP VSRIDLSRLA QLAPASITKI VHEMLEAHLV QELEIKEAGN RGRPAVGLVV
ETEAWHYLSL RISRGEIFLA LRDLSSKLVV EESQELALKD DLPLLDRIIS HIDQFFIRHQ KKLERLTSIA ITLPGIIDTE
NGIVHRMPFY EDVKEMPLGE ALEQHTGVPV YIQHDISAWT MAEALFGASR GARDVIQVVI DHNVGAGVIT DGHLLHAGSS
SLVEIGHTQV DPYGKRCYCG NHGCLETIAS VDSILELAQL RLNQSMSSML HGQPLTVDSL CQAALRGDLL AKDIITGVGA
HVGRILAIMV NLFNPQKILI GSPLSKAADI LFPVISDSIR QQALPAYSQH ISVESTQFSN QGTMAGAALV KDAMYNGSLL
IRLLQG
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B4. Purification Strategy/Source

University of Potsdam (Prof. Heiko Möller)

B5. Stock Concentration/Stock Buffer

4.45 mg/mL | 100 µM
10 mM HEPES, pH 7.2, 150 mM NaCl, 10 mM KCl, 1 mM $MgCl_2$, 0.5 mM EDTA

B6. Molecular Weight/Extinction Coefficient

44.5 kDa
23,170 $M^{-1}cm^{-1}$ (ε_{280})

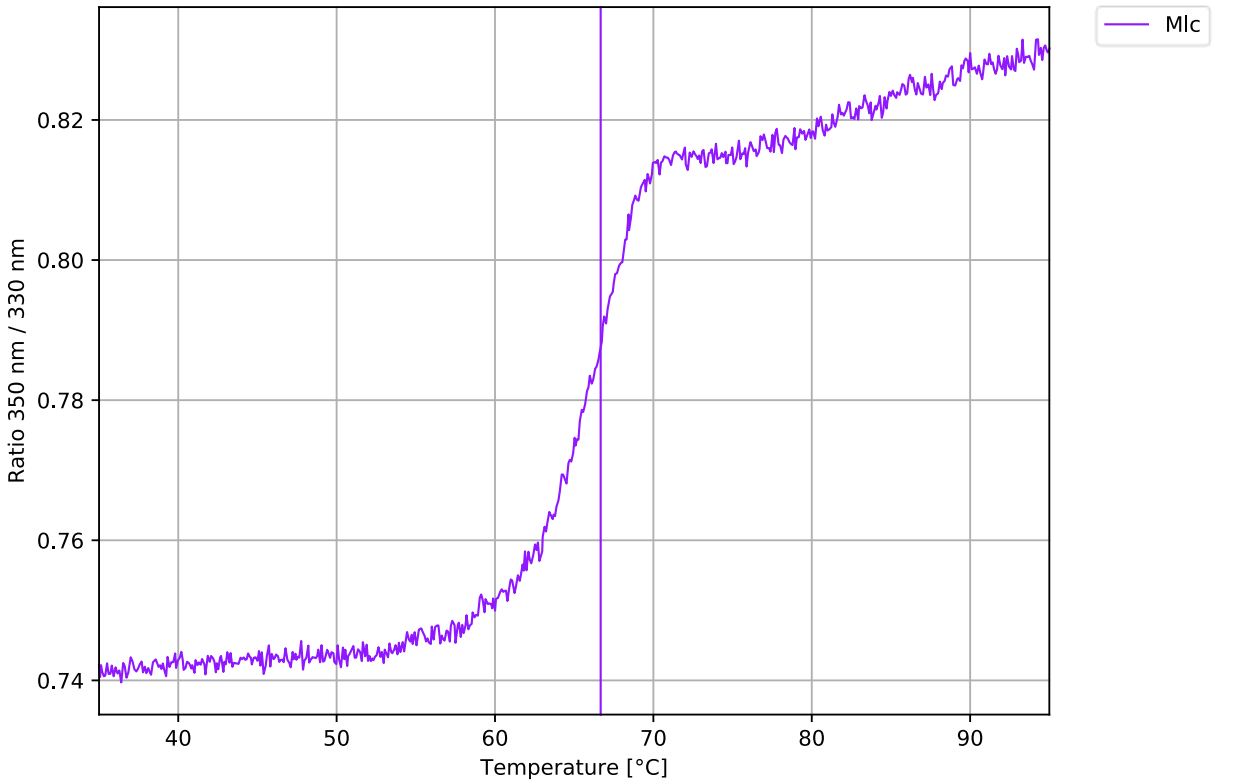
B7. Serial Dilution Preparation

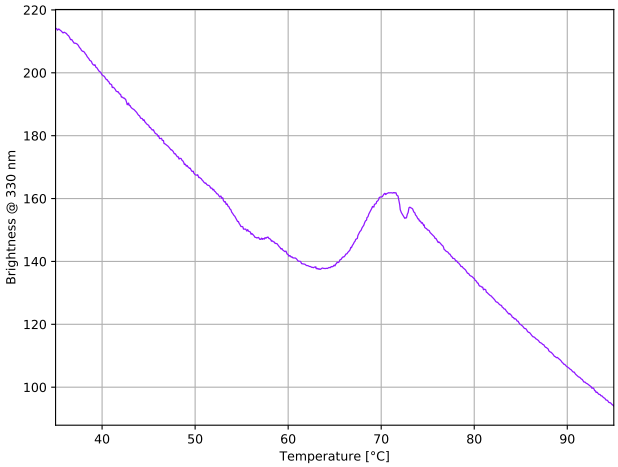
1. Mix 3 µL of 100 µM Mlc (monomer) with 27 µL of dilution buffer to obtain 30 µL of a 20 µM Mlc solution.
2. Mix 2 µL of 1 µM BoxP1 Hairpin with 198 µL of dilution buffer to obtain 200 µL of 10 nM BoxP1 Hairpin.
3. Take a fresh 0.5 mL tube and mix 160 µL of 10 nM BoxP1 Hairpin with 160 µL of dilution buffer to obtain 320 µL of a 5 nM BoxP1 Hairpin solution.
4. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 5 nM BoxP1 Hairpin solution into tubes **2** to **16**. Then, mix 20 µL of 20 µM Mlc with 20 µL of 10 nM BoxP1 Hairpin in tube **1**.
5. Prepare a 1:1 serial dilution by transferring 20 µL from tube to tube. Mix carefully by pipetting up and down.
6. Incubate for 10 minutes at room temperature in the dark before loading capillaries.

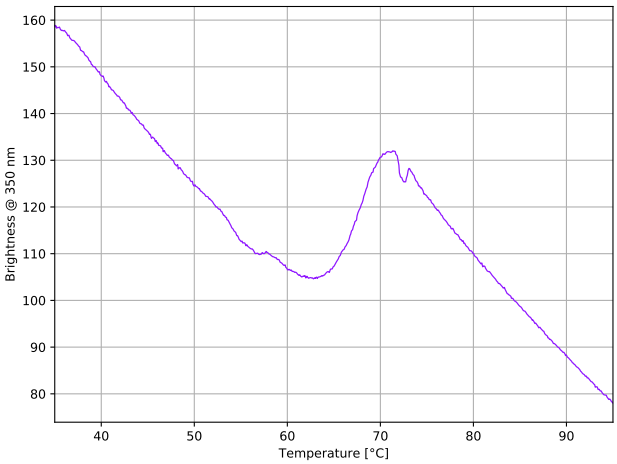
C. Tycho

Validation of structural integrity of Mlc using Tycho NT.6:
nanotempertech.com/tycho

Mlc	10 µL of 20 µM Mlc (monomer)	T_i = 66.7°C







D1. Monolith System/Capillaries

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

D2. Monolith Software

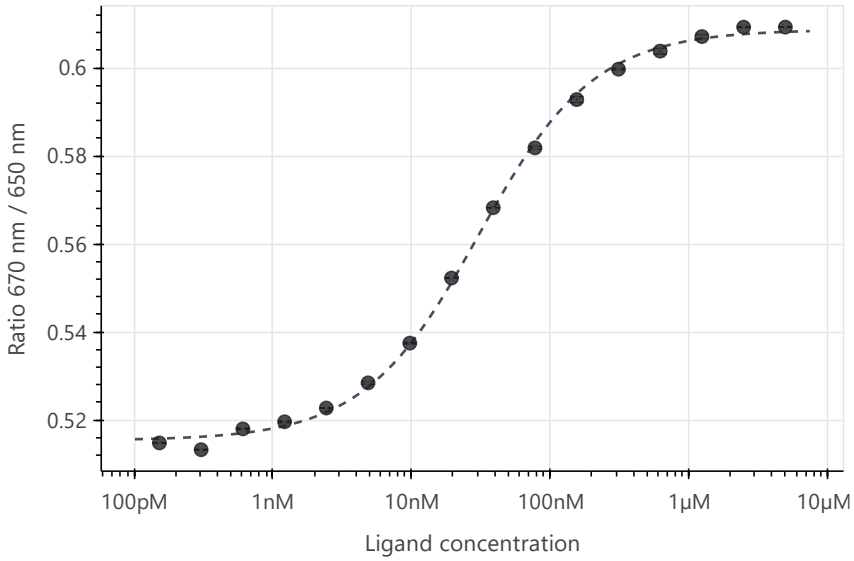
MO.Control v2.4.2 (NanoTemper Technologies GmbH)
https://nanotempertech.com/monolith-mo-control-software/

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

10 mM HEPES, pH 7.2, 150 mM NaCl, 10 mM KCl, 1 mM $MgCl_2$, 0.5 mM EDTA
5 nM BoxP1 | 5 µM – 0.15 nM Mlc (dimer[1]) | 20°C | 100% excitation power

D4. Monolith Results (Dose Response)

K_d = 26.4 nM ± 1.2 (S/N = 73.4)



D5. Reference Results/Supporting Results

K_d = 24 nM	Surface Plasmon Resonance (SPR) Dissertation David Witte, University of Konstanz, 2015
K_d = 29.8 nM	Isothermal Titration Calorimetry (ITC) Dissertation David Witte, University of Konstanz, 2015

E. Contributors

David Witte[2], Andreas Langer[3]

[1] A highest Mlc monomer concentration of 10 µM corresponds to a 5 µM functional dimer concentration.
[2] Proteros Biostructures GmbH, Planegg, Germany
[3] NanoTemper Technologies GmbH, München, Germany | nanotempertech.com