Monolith Protocol MO-P-024

BoxP1 Hairpin DNA – Mlc

Mlc (*makes large colonies*) from *Escherichia coli* is a transcriptional repressor which controls the expression of a number of 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 – DNA interaction | 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

N/A

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

5’-Cy5 labeled

A9. Labeling Procedure

N/A

A10. Labeling Efficiency

HPLC-purified, 100% labeled DNA

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})

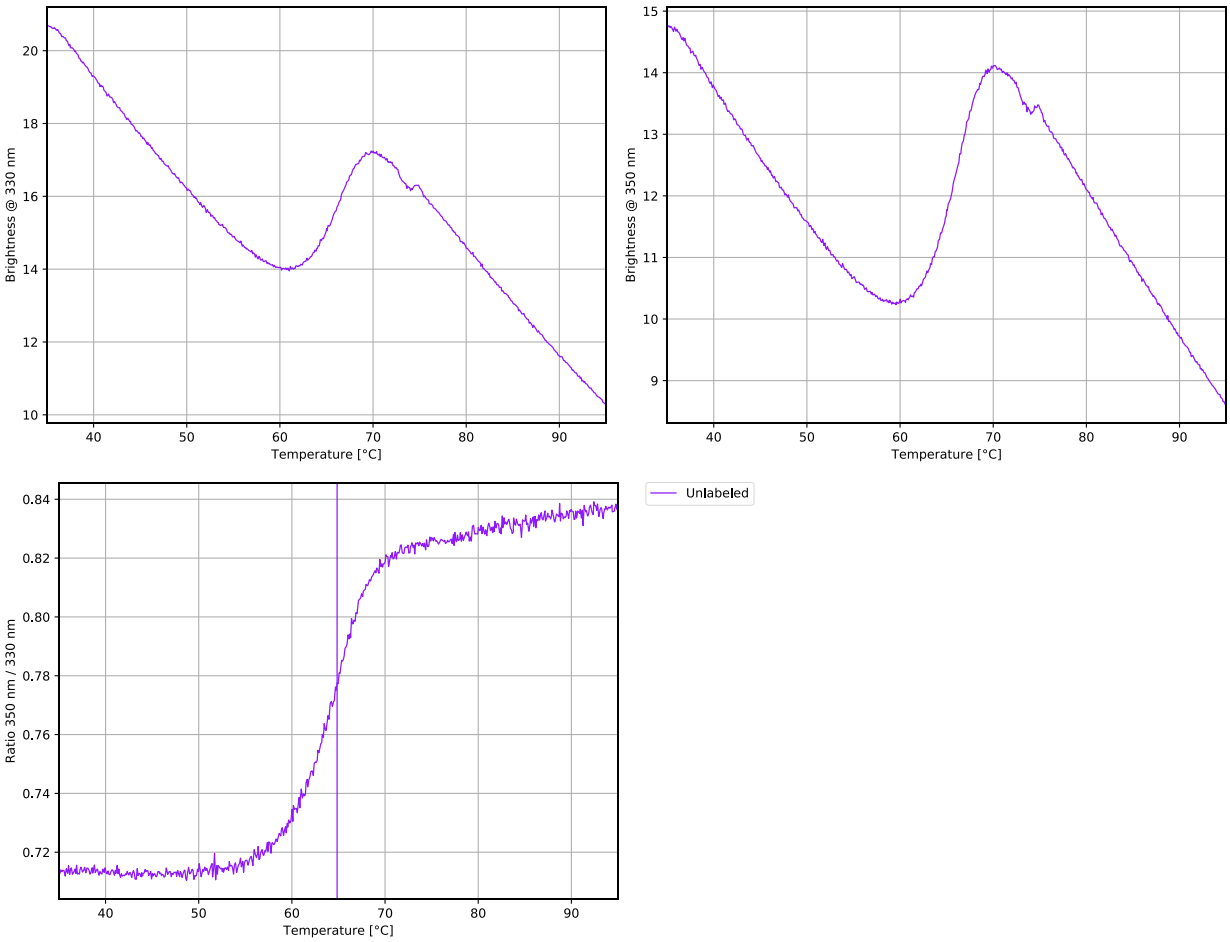
B7. Serial Dilution Preparation

1. Add 2 µL of Mlc to 48 µL of dilution buffer to obtain 50 µL of a 4 µM Mlc solution.
2. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 4 µM Mlc solution 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. Mix 2 µL of 300 µM BoxP1 Hairpin with 1.2 mL of dilution buffer to obtain a 500 nM BoxP1 Hairpin solution.
5. Mix 2 µL of 500 nM BoxP1 Hairpin with 198 µL of dilution buffer to obtain 200 µL of 5 nM BoxP1 Hairpin.
6. Add 10 µL of 5 nM BoxP1 Hairpin to each tube from **16** to **1** and mix by pipetting.
7. Incubate for 20 minutes at room temperature in the dark before loading capillaries.

C. Applied Quality Checks

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

Unlabeled	10 µL of 2 µM Mlc	T_i = 64.2°C



D1. MST System/Capillaries

Monolith NT.115 Red (NanoTemper Technologies GmbH)
Premium Capillaries Monolith NT.115 (MO-K025, NanoTemper Technologies GmbH)

D2. MST Software

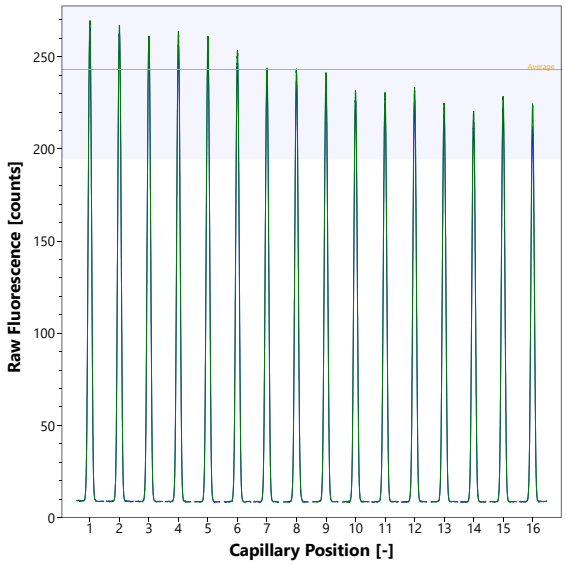
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

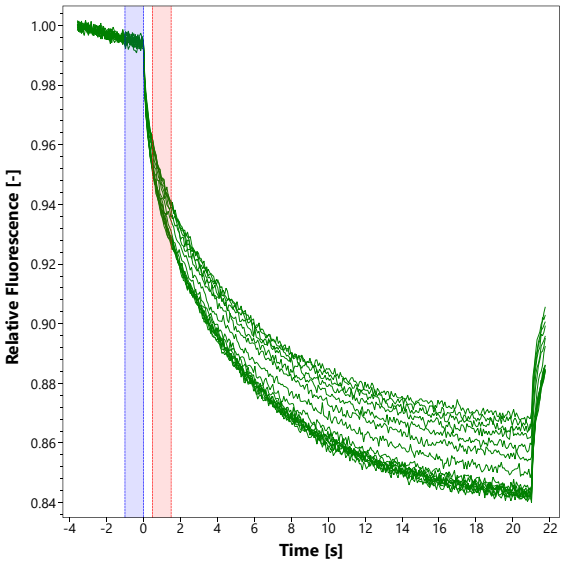
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

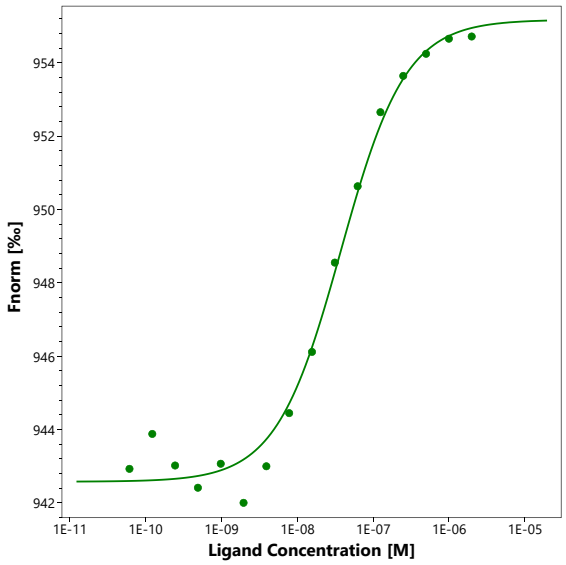
10 mM HEPES, pH 7.2, 150 mM NaCl, 10 mM KCl, 1 mM $MgCl_2$, 0.5 mM EDTA
2.5 nM BoxP1 | 2 µM – 60 pM Mlc | 25°C | medium MST power | 80% excitation power

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

K_d = 36.3 nM







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

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