Monolith Protocol MO-P-025

Dimerization of Mlc Homodimers

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. Hence, above a certain Mlc concentration, two homodimers can dimerize into a tetramer.

protein dimerization | homodimer | tetramer

A1. Target/Fluorescent Molecule

Mlc
uniprot.org/uniprot/P50456

A2. Molecule Class/Organism

Transcriptional repressor protein
Escherichia coli

A3. Sequence/Formula

```
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
```

A4. Purification Strategy/Source

University of Potsdam (Prof. Heiko Möller)

A5. 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

A6. Molecular Weight/Extinction Coefficient

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

A7. Dilution Buffer

10 mM HEPES, pH 7.4, 150 mM NaCl, 0.005% TWEEN® 20

A8. Labeling Strategy

Monolith Protein Labeling Kit RED – NHS 2nd Generation (MO-L011, NanoTemper Technologies GmbH)
1* Labeling Buffer NHS | 1* Dye RED-NHS 2nd Generation (10 µg) | 1* B-Column

A9. Labeling Procedure

1. Add 90 µL of Labeling Buffer NHS to 10 µL of 100 µM Mlc to obtain 100 µL of a 10 µM solution.
2. Add 25 µL of DMSO to 10 µg RED-NHS 2nd Generation dye to obtain a ~600 µM solution. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
3. Mix 6.7 µL of the 600 µM dye solution with 93.3 µL of Labeling Buffer NHS to obtain 100 µL of a 40 µM dye solution (4x protein concentration).
4. Mix Mlc and dye in a 1:1 volume ratio (200 µL final volume, 3.3% final DMSO concentration).
5. Incubate for 30 minutes at room temperature in the dark.
6. In the meantime, remove the top cap of the B-Column and pour off the storage solution. Remove the bottom cap and place with adapter in a 15 mL tube.
7. Fill the column with dilution buffer and allow it to enter the packed resin bed completely by gravity flow. Discard the flow through collected. Repeat this step 3 more times.
8. Add 200 µL of the labeling reaction from step 4 to the center of the column and let sample enter the resin bed completely.
9. Add 500 µL of dilution buffer after the sample has entered and discard the flow through.
10. Place column in a new collection tube, add 250 µL of dilution buffer and collect the eluate.
11. Keep the labeled Mlc (~4 µM) on ice in the dark.

A10. Labeling Efficiency

Measurement of protein concentration and degree of labeling (DOL) using a NanoDrop™:
nanotempertech.com/dol-calculator

Absorbance A_{280}	0.094	Protein concentration	3.27 µM
Absorbance A_{650}	0.454	Degree-of-labeling (DOL)	0.71

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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University of Potsdam (Prof. Heiko Möller)

B5. Stock Concentration/Stock Buffer

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B6. Molecular Weight/Extinction Coefficient

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B7. Serial Dilution Preparation

1. Add 2 µL of unlabeled Mlc to 48 µL of dilution buffer to obtain 50 µL of a 4 µM 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 labeled Mlc with 198 µL of dilution buffer to obtain 200 µL of ~40 nM Mlc.
5. Mix 10 µL of ~40 nM labeled Mlc with 190 µL of dilution buffer to obtain 200 µL of ~2 nM Mlc.
6. Add 10 µL of labeled Mlc (~2 nM) 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.

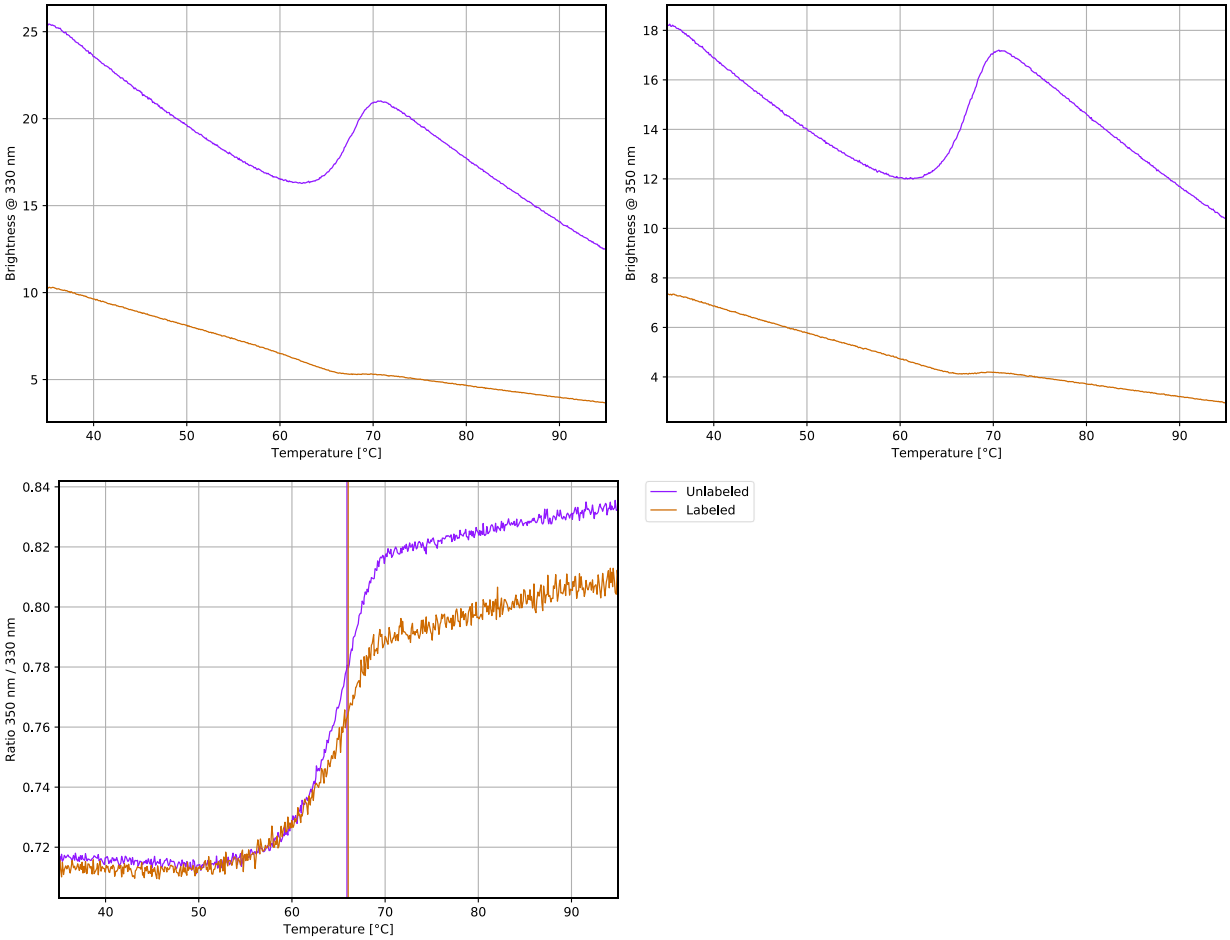
B8. SD-Test

1. Centrifuge the remainder of tubes **1** to **3** and **14** to **16** for 10 minutes at 15.000 × g.
2. Prepare the SD-mix: Dilute 400 µL of 10% SDS and 40 µL of 1 M DTT in 560 µL of water to obtain a solution containing 4% SDS and 40 mM DTT.
3. Transfer 7 µL of the SD-mix to six PCR tubes.
4. Add 7 µL from the remainder of tubes **1** to **3** and **14** to **16** to the tubes containing 7 µL SD-mix. Mix well by pipetting.
5. Place samples on a heat block set to 95°C for 5 minutes to denature the protein, then allow to cool at 25°C for 10 minutes before loading into capillaries.

C. Applied Quality Checks

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

Unlabeled	10 µL of 4 µM Mlc	T_i = 65.9°C
Labeled	10 µL of B-column eluate (~4 µM)	T_i = 66.0°C



D1. MST System/Capillaries

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

D2. MST Software

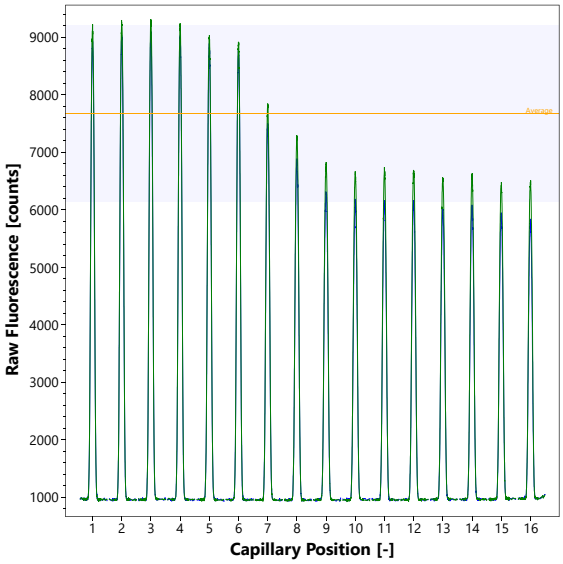
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

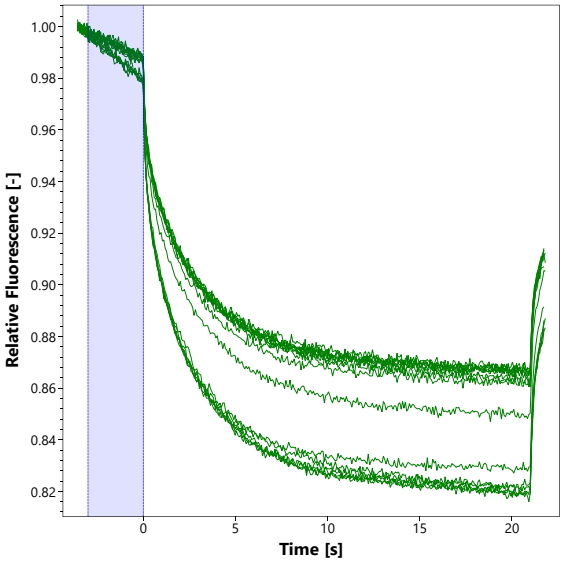
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

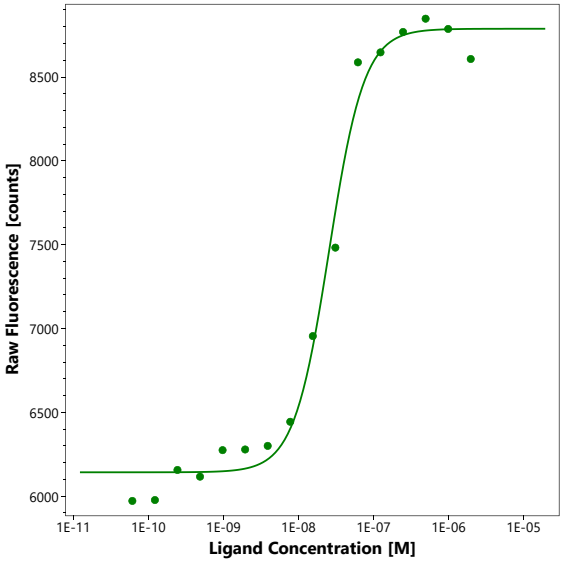
10 mM HEPES, pH 7.4, 150 mM NaCl, 0.005% TWEEN® 20
1 nM (labeled) Mlc | 2 µM – 60 pM (unlabeled) Mlc | 25°C | medium MST power | 100% excitation power

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

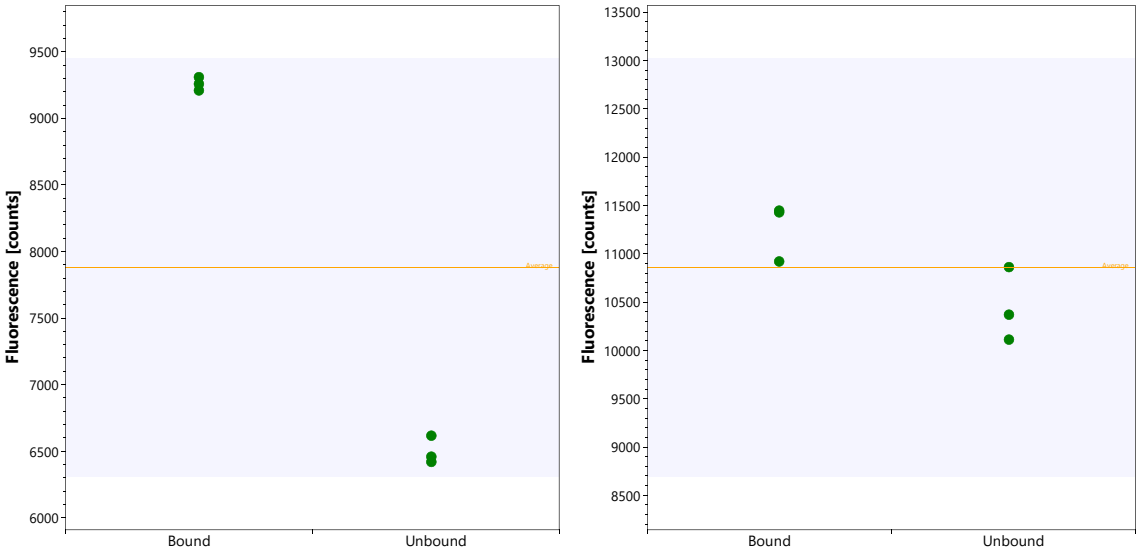
EC_{50} = 25.8 nM







SD-Test:



D5. Reference Results/Supporting Results

[…] there exists an equilibrium between the dimeric and the tetrameric form of Mlc and […] the crystallization buffer (1.6 M $MgSO_4$, 100 mM MES, pH 6.5) shifts the equilibrium toward the dimeric state of Mlc, whereas the buffer used in size exclusion chromatography (300 mM NaCl, 50 mM Tris-HCl, pH 7.5) favors the tetrameric state.
Schiefner et al., J Biol Chemistry 280 (32), 29073–29079 (2005)

E. Contributors

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