NANOTEMPER

Monolith Protocol MO-P-036

Calmodulin – Ca^{2+}

Calmodulin (CaM) is the primary receptor for calcium ions in human cells, acting as a mediator of calcium functions and regulating many cellular processes. Depending on its conformational state, CaM binds or regulates a multitude of protein targets. On binding to calcium, CaM undergoes a large conformational change.

protein – ion interaction | calcium

A1. Target/Fluorescent Molecule

Calmodulin bovine (CaM)
uniprot.org/uniprot/P62157

A2. Molecule Class/Organism

Calcium-binding messenger protein
Bos taurus (Bovine)

A3. Sequence/Formula

MGSSHHHHHH SSGLVPRGSH MADQLTEEQI AEFKEAFSLF DKDGDGTITT KELGTVMRSL GQNPTEAELQ DMINEVDADG NGTIDFPEFL TMMARKMKDT DSEEEIREAF RVFDKDGNGY ISAAELRHVM TNLGEKLTDE EVDEMIREAD IDGDGQVNYE EFVQMMTAK

A4. Purification Strategy/Source

Recombinant, expressed in E. coli, His_6-tagged
Sigma Aldrich GmbH
C4874

A5. Stock Concentration/Stock Buffer

1 mg/mL | 52.6 µM

A6. Molecular Weight/Extinction Coefficient

19.0 kDa
2,980 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

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

A8. Labeling Strategy

Monolith His-Tag Labeling Kit RED-tris-NTA 2nd Generation (MO-L018, NanoTemper Technologies GmbH)
1* 125 pmol RED-tris-NTA Dye 2nd Generation

A9. Labeling Procedure

1. Prepare a 100 µM EDTA solution in dilution buffer.
2. Mix 12.1 µL of dilution buffer with 2.9 µL of 52.6 µM CaM to obtain 15 µL of a 10 µM CaM solution.
3. Mix 4 µL of dilution buffer with 4 µL of 10 µM CaM and 2 µL of 100 µM EDTA to obtain 10 µL of a 4 µM CaM solution in dilution buffer containing 20 µM EDTA (5-fold excess[1]).
4. Incubate for 5 minutes to assure that all Ca^{2+}-ions are removed from CaM by EDTA.
5. Add 90 µL of dilution buffer to the solution of step 3 to obtain 100 µL of a 400 nM CaM solution in dilution buffer containing 2 µM EDTA.
6. Suspend 125 pmol RED-tris-NTA Dye 2nd Generation in 25 µL of dilution buffer to obtain a 5 µM dye solution.
7. Mix 123 µL of dilution buffer with 2 µL dye (5 µM) to obtain 125 µL of a 80 nM dye solution.
8. Add 100 µL of the dye solution (80 nM) to the solution of step 5 to obtain 200 µL of a 200 nM CaM, 40 nM dye solution in dilution buffer containing 1 µM EDTA[2].
9. Incubate for 20 minutes at room temperature in the dark.

A10. Labeling Efficiency

N/A

B1. Ligand/Non-Fluorescent Binding Partner

Calcium ion (Ca^{2+})

B2. Molecule Class/Organism

Divalent metal ion

B3. Sequence/Formula

Ca^{2+}

B4. Purification Strategy/Source

Calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$)
Carl Roth GmbH
T885.2

[1] A 5-fold excess of EDTA is used, as CaM can bind up to 4 Ca^{2+}-ions.
[2] High concentrations of EDTA are not recommended when using the His-Tag Labeling Kit, as EDTA can chelate Ni^{2+}-ions from tris-NTA. At a concentration of only 1 µM EDTA, however, labeling of proteins remains stable over several hours. Furthermore, the bound His-tag helps to ‘protect’ the Ni^{2+}-ions on tris-NTA from removal by EDTA.

B5. Stock Concentration/Stock Buffer

Powdered

B6. Molecular Weight/Extinction Coefficient

40 Da

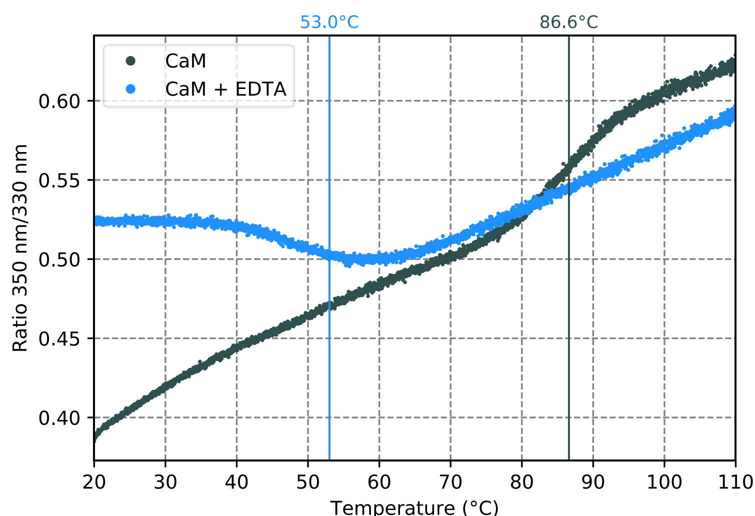
B7. Serial Dilution Preparation

1. Dissolve 73.5 mg of calcium chloride dihydrate (MW: 147,02 g/mol) in 10 mL of dilution buffer to obtain a 50 mM $CaCl_2$ stock solution.
2. Mix 2 µL of the 50 mM $CaCl_2$ stock with 198 µL of dilution buffer to obtain 200 µL of a 500 µM $CaCl_2$ solution.
3. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 1 mM $CaCl_2$ solution into tube **1**. Then, transfer 10 µL of dilution buffer into tubes **2** to **16**.
4. 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.
5. Add 10 µL of labeled CaM (~200 nM) to each tube from **16** to **1** and mix by pipetting.
6. Incubate for 5 minutes in the dark before loading capillaries.

C. Applied Quality Checks

Validation of structural integrity and functionality of CaM via a thermal shift assay (1°C/min) with EDTA:
nanotempertech.com/prometheus

CaM	5 µL of 10 µM CaM + 5 µL of dilution buffer	T_m = 86.6°C
CaM + EDTA	5 µL of 10 µM CaM + 5 µL of 100 µM EDTA	T_m = 53.0°C[3]



[3] As CaM is already complexed with Ca^{2+} in storage buffer, it needs to be removed prior to the MST experiment (c.f. A9, step 4). Since CaM does **not** contain any Tryptophans, unfolding of the Ca^{2+}-free form shows a 'blue-shift', while the conformational change upon unfolding of the Ca^{2+}-bound form results in a 'red-shift'.

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

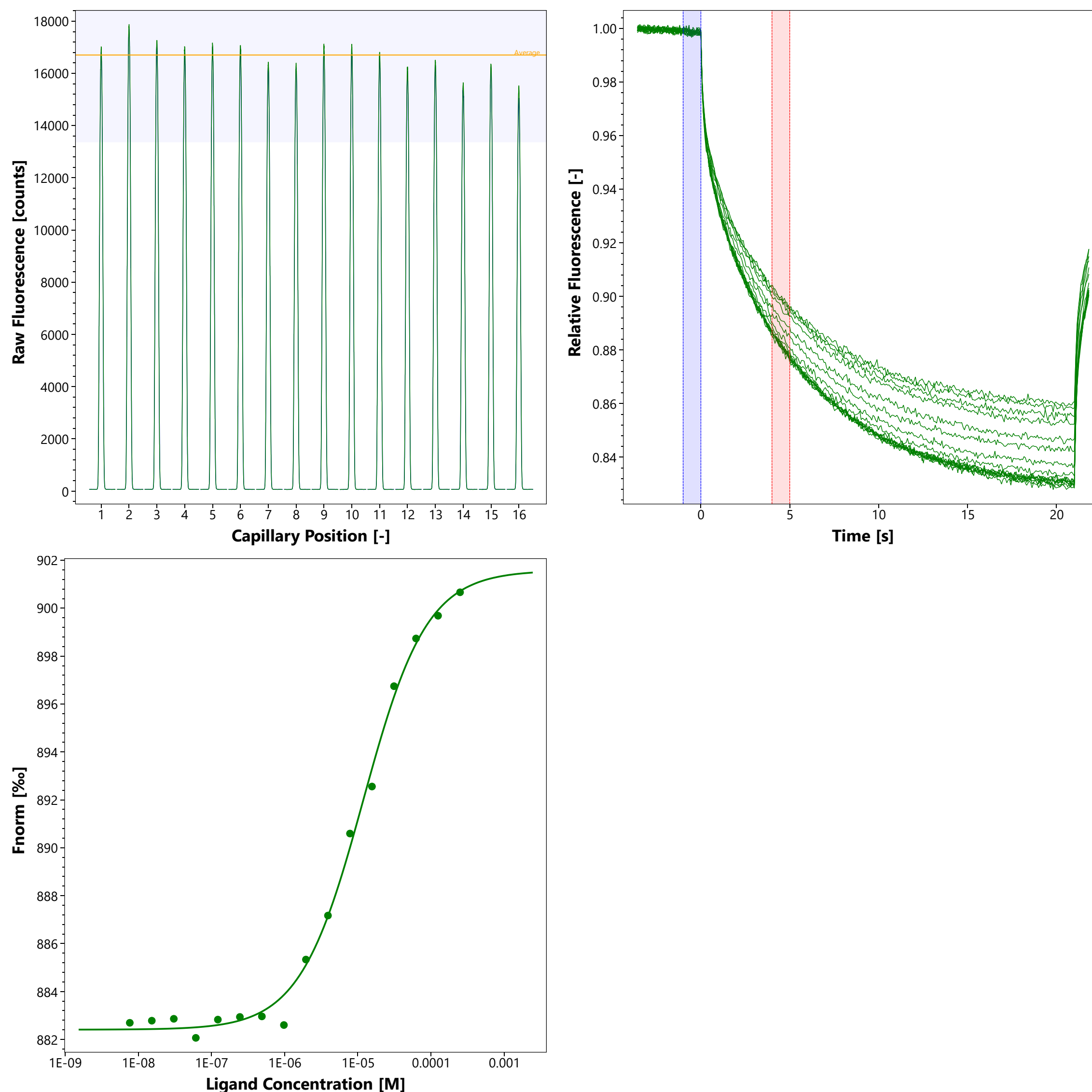
MO.Control v1.6 (NanoTemper Technologies GmbH)
https://nanotempertech.com/monolith-mo-control-software/

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

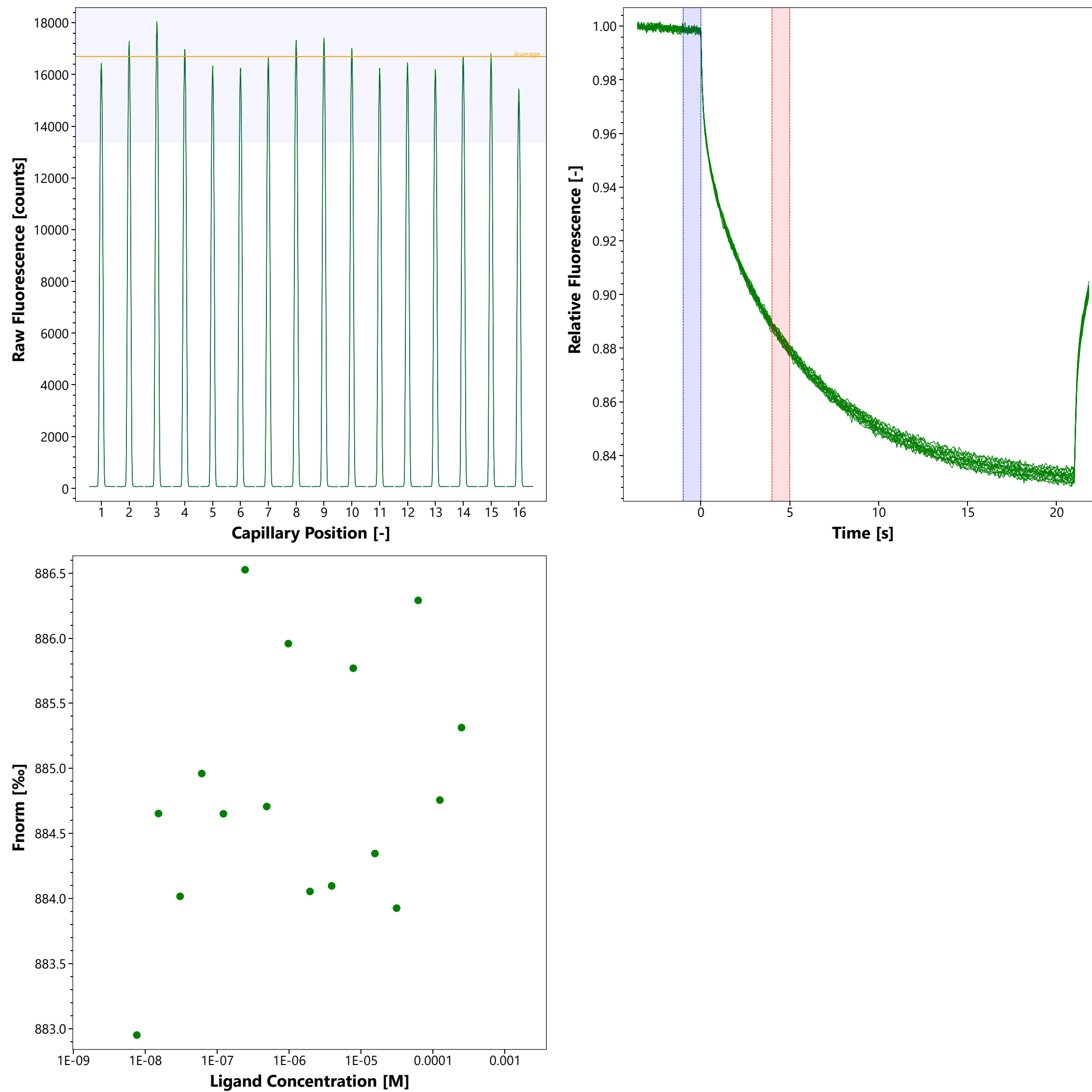
20 mM HEPES, pH 7.4, 150 mM NaCl, 0.5 µM EDTA, 0.005% TWEEN® 20
100 nM CaM | 250 µM – 7.6 nM $CaCl_2$ | 25°C | medium MST power | 5% excitation power

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

K_d = 11.8 µM



Negative control: $MgCl_2$ instead of $CaCl_2$



D5. Reference Results/Supporting Results

$K_d = 2 – 50\ \mu M$	Tyrosine fluorescence changes Tsuruta and Sano, Biophysical Chemistry, 35, 75-84 (1990)
$T_{1/2} = 55°C$ (w/ EDTA), $T_{1/2} > 90°C$ (w/ Ca^{2+})	Circular Dichroism Brzeska et al., FEBS Letters 153 (1), 169-173 (1983)

E. Contributors

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