Monolith Protocol MO-P-020

Sirtuin 5 – Compound

Sirtuin 5 (also known as Sirt5) is a NAD-dependent demalonylase, desuccinylase and deglutarylase that specifically removes malonyl, succinyl and glutaryl groups from lysine residues of target proteins. It activates carbamoyl phosphate synthetase (CPS1) and contributes to the regulation of blood ammonia levels during prolonged fasting, thereby increasing CPS1 activity in response to elevated NAD levels.

protein – small molecule interaction | deacylase | inhibitor | His_6-tag

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A1. Target/Fluorescent Molecule

Sirtuin 5 (Sirt5)
uniprot.org/uniprot/Q9NXA8

A2. Molecule Class/Organism

NAD-dependent protein deacylase
Homo sapiens (Human)

A3. Sequence/Formula

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MRPLQIVPSR LISQLYCGLK PPASTRNQIC LKMARPSSSM ADFRKFFAKA KHIVIISGAG VSAESGVPTF
RGAGGYWRKW QAQDLATPLA FAHNPSRVWE FYHYRREVMG SKEPNAGHRA IAECETRLGK QGRRVVVITQ
NIDELHRKAG TKNLLEIHGS LFKTRCTSCG VVAENYKSPI CPALSGKGAP EPGTQDASIP VEKLPRCEEA
GCGGLLRPHV VWFGENLDPA ILEEVDRELA HCDLCLVVGT SSVVYPAAMF APQVAARGVP VAEFNTETTP
ATNRFRFHFQ GPCGTTLPEA LACHENETVS
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A4. Purification Strategy/Source

N/A

A5. Stock Concentration/Stock Buffer

7.2 mg/mL | 213 µM

A6. Molecular Weight/Extinction Coefficient

33.8 kDa
30,940 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

Phosphate-buffered saline (PBS, pH 7.4), 0.05% TWEEN® 20

A8. Labeling Strategy

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

A9. Labeling Procedure

1. Add 105 µL of dilution buffer to 2 µL of 213 µM Sirt5 to obtain 107 µL of a 4 µM solution.
2. Suspend 125 pmol RED-tris-NTA Dye 2nd Generation in 25 µL of dilution buffer to obtain a 5 µM dye solution.
3. Mix 193 µL of dilution buffer with 2 µL dye (5 µM) and 5 µL Sirt5 (4 µM) to obtain 200 µL of a 100 nM Sirt5, 50 nM dye solution.
4. Incubate for 30 minutes at room temperature in the dark.

A10. Labeling Efficiency

N/A

B1. Ligand/Non-Fluorescent Binding Partner

Compound

B2. Molecule Class/Organism

Small molecule compound

B3. Sequence/Formula

N/A

B4. Purification Strategy/Source

N/A

B5. Stock Concentration/Stock Buffer

1 mM
DMSO

B6. Molecular Weight/Extinction Coefficient

N/A

[1] Both, NHS- and Maleimide labeling, are not recommended for sirtuins since lysines are important for the interaction with the substrate/ligand while cysteines are structurally important.

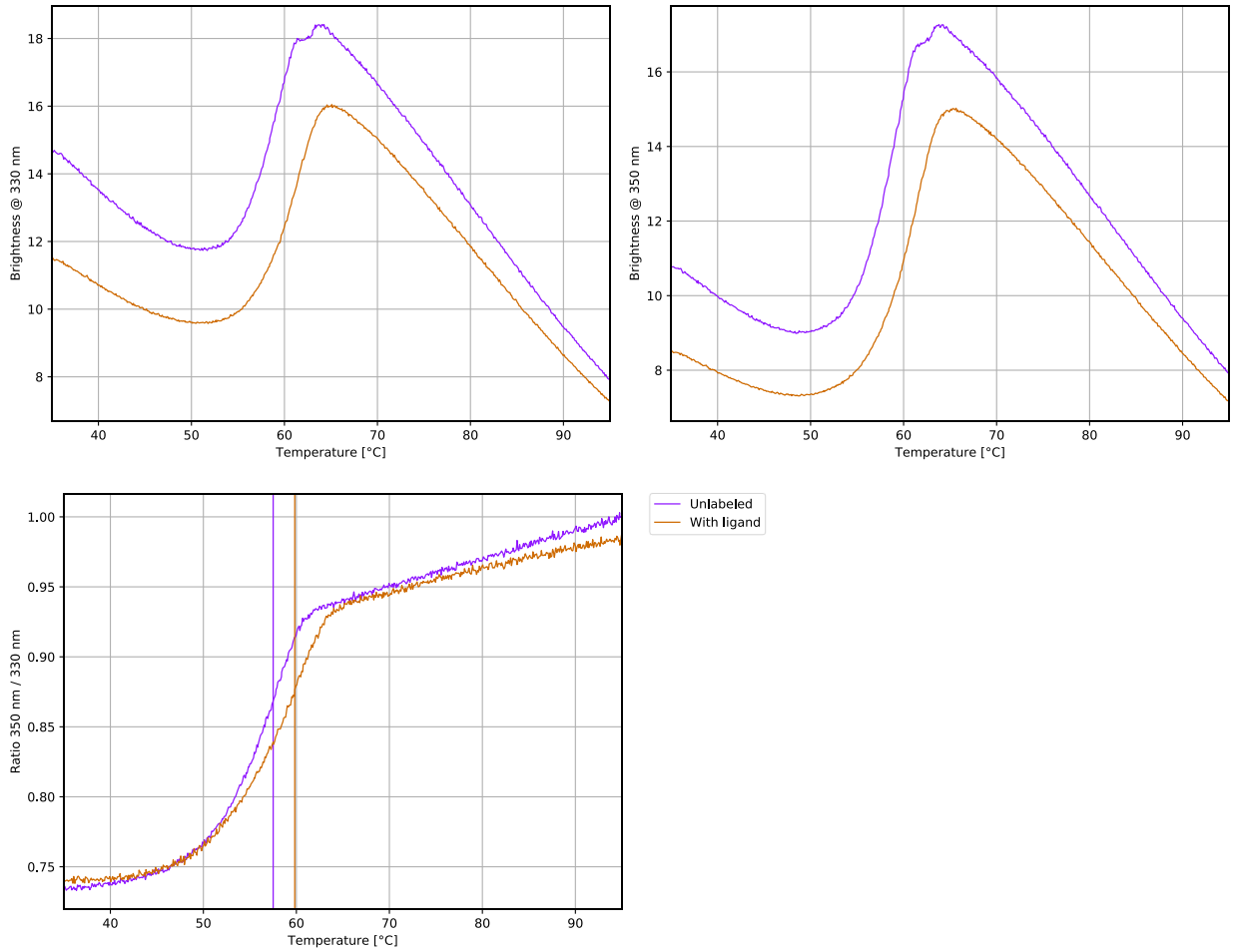
B7. Serial Dilution Preparation

1. Add 98 µL of dilution buffer to 2 µL of 1 mM compound to obtain 100 µL of a 20 µM.
2. Mix 4 µL of DMSO with 196 µL of dilution buffer to obtain 200 µL of a 2% DMSO solution.
3. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 20 µM compound solution into tube **1**. Then, transfer 10 µL of the 2% DMSO solution 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 Sirt5 (100 nM) to each tube from **16** to **1** and mix by pipetting.

C. Applied Quality Checks

Validation of structural integrity and functionality of labeled Sirt5 using Tycho NT.6:
nanotempertech.com/tycho

Unlabeled	5 µL of 4 µM Sirt5 + 5 µL of dilution buffer containing 2% DMSO	T_i = 57.5°C
With ligand	5 µL of 4 µM Sirt5 + 5 µL of 20 µM compound	T_i = 59.8°C



D1. MST System/Capillaries

Monolith NT.115 Red (NanoTemper Technologies GmbH)
Capillaries Monolith NT.115 (MO-K022, NanoTemper Technologies GmbH)

D2. MST Software

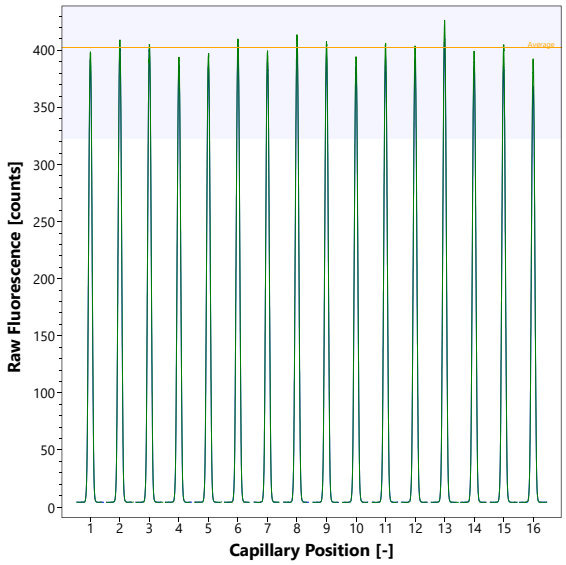
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

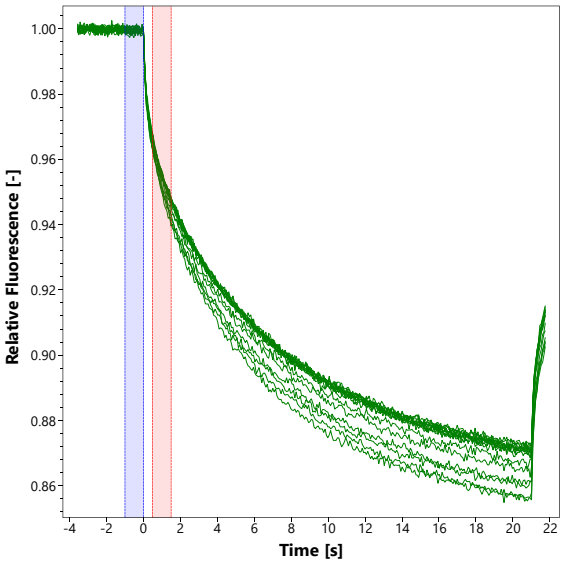
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

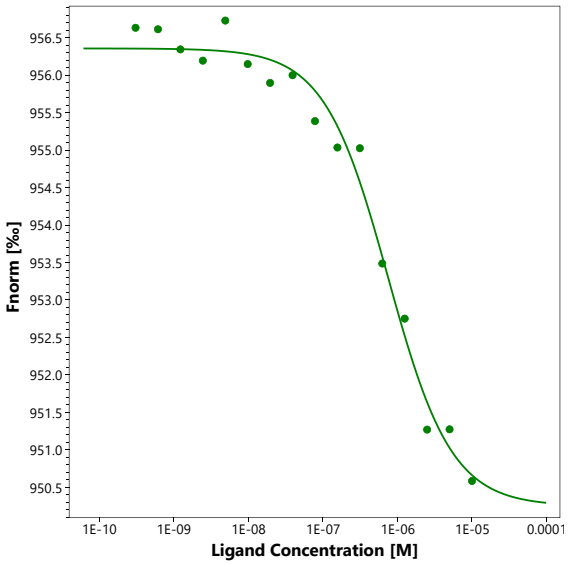
Phosphate-buffered saline (PBS, pH 7.4), 0.05% TWEEN® 20, 1% DMSO
50 nM Sirt5 | 10 µM – 305 pM compound | 25°C | medium MST power | 40% excitation power

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

K_d = 722 nM







D5. Reference Results/Supporting Results

N/A

E. Contributors

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