Monolith Protocol MO-P-055

STING – 2',3'-cGAMP

Stimulator of interferon genes (STING) is a protein that plays an important role in innate immunity. STING induces type I interferon production when cells are infected with intracellular pathogens, such as viruses, mycobacteria and intracellular parasites. Innate immune response is triggered in response to non-CpG double-stranded DNA from viruses and bacteria delivered to the cytoplasm. STING acts by binding cyclic dinucleotides. It recognizes and binds cyclic di-GMP (c-di-GMP), a second messenger produced by bacteria, and cyclic GMP-AMP (cGAMP), a messenger produced by CGAS in response to DNA virus in the cytosol.

protein – small molecule interaction | innate immune response | His_6-tag

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A1. Target/Fluorescent Molecule

Stimulator of interferon genes (STING)
uniprot.org/uniprot/Q86WV6

A2. Molecule Class/Organism

Transmembrane protein
Homo sapiens (Human)

A3. Sequence/Formula

N/A

A4. Purification Strategy/Source

His_6-tagged
Crelux GmbH

A5. Stock Concentration/Stock Buffer

5.8 mg/mL | 17.7 µM
20 mM HEPES/NaOH, pH 8.0, 150 mM NaCl, 0.5 mM TCEP

A6. Molecular Weight/Extinction Coefficient

32.8 kDa
33,350 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

50 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM DTT, 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

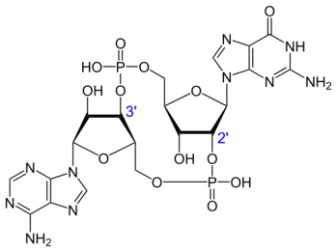
1. Suspend 125 pmol RED-tris-NTA Dye 2nd Generation in 25 µL PBS-T to obtain a 5 µM dye solution.
2. Mix 590 µL of dilution buffer with 3 µL dye (5 µM) and 6.8 µL STING (17.7 µM) to obtain 600 µL of a 200 nM STING, 25 nM dye solution.
3. Incubate for 30 minutes at room temperature in the dark.
4. Centrifuge for 10 min at 4°C and 15,000 × g.

A10. Labeling Efficiency

N/A

B1. Ligand/Non-Fluorescent Binding Partner

Cyclic guanosine monophosphate–adenosine monophosphate (2',3'-cGAMP)



B2. Molecule Class/Organism

Cyclic dinucleotide

B3. Sequence/Formula

$C_{20}H_{24}N_{10}O_{13}P_2$

B4. Purification Strategy/Source

Sigma-Aldrich GmbH
SML1229

B5. Stock Concentration/Stock Buffer

100 µM
ddH_2O

B6. Molecular Weight/Extinction Coefficient

674.41 Da

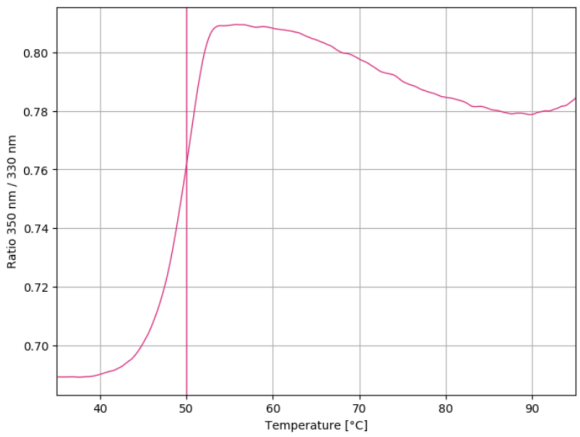
B7. Serial Dilution Preparation

1. Mix 16 µL of DMSO with 184 µL of dilution buffer to obtain 200 µL of a 8% DMSO solution.
2. Prepare a PCR-rack with 16 PCR tubes. Mix 4 µL of the 100 µM 2',3'-cGAMP solution with 14.4 µL of dilution buffer and 1.6 µL of DMSO in tube **1**. Then, transfer 10 µL of the 8% DMSO solution 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. Add 10 µL of labeled STING (200 nM) to each tube from **16** to **1** and mix by pipetting.
5. Incubate for 30 minutes at room temperature in the dark before loading capillaries.

C. Applied Quality Checks

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

STING	10 µL of 17.7 µM STING in dilution buffer	T_i = 50.0°C



D1. MST System/Capillaries

Monolith NT.Automated, picoRED detector (NanoTemper Technologies GmbH)
Premium Capillary Chips Monolith NT.Automated (MO-AK005, NanoTemper Technologies GmbH)

D2. MST Software

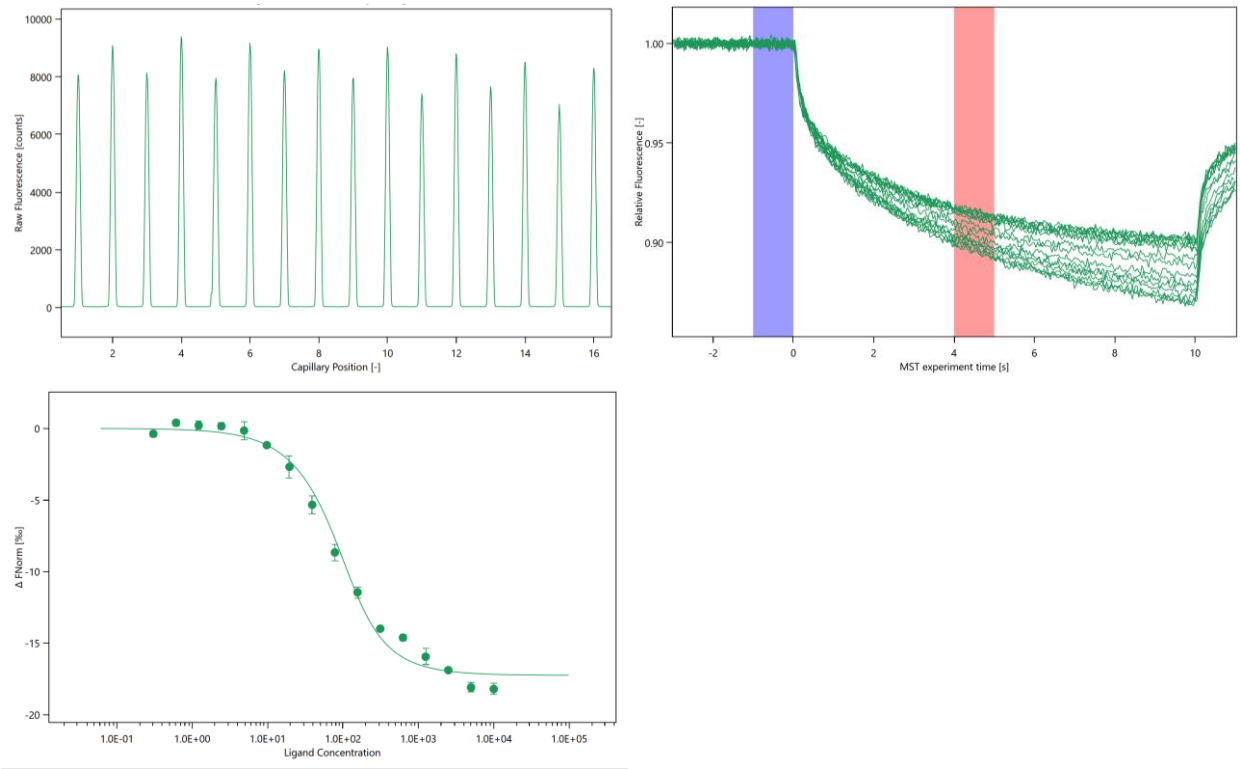
MO.ScreeningControl v1.10 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith

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

50 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM DTT, 0.005% TWEEN® 20, 4% DMSO
100 nM STING | 10 µM – 0.31 nM 2',3'-cGAMP | 25°C | medium MST power | 5% excitation power

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

K_d = 41.5 nM



D5. Reference Results/Supporting Results

K_d = 3.79 nM Isothermal Titration Calorimetry (ITC)
Zhang et al., Molecular Cell 51 (2013) 1–10

E. Contributors

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