Monolith Protocol MO-P-060

Lysozyme – NAG_3 (no column purification)

Lysozyme is an enzyme that prevents bacterial infections by attacking peptidoglycan, a component of certain bacterial cell walls. Peptidoglycan is composed of the repeating amino sugars N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), which are crosslinked by peptide bridges. Lysozyme hydrolyzes the bond between NAG and NAM, increasing the bacteria's permeability and causing the bacteria to burst. It is widely distributed in plants and animals. The majority of the lysozyme used in research is purified from hen egg whites. Tri-N-acetyl-D-glucosamine (NAG_3) is an inhibitor of lysozyme.

protein – small molecule | carbohydrate

A1. Target/Fluorescent Molecule

Lysozyme
uniprot.org/uniprot/B8YK79

A2. Molecule Class/Organism

Glycoside hydrolase
Gallus gallus (Chicken)

A3. Sequence/Formula

```
KVFGRCELAA AMKRHGLDNY RGYSLGNWVC AAKFESNFNT QATNRNTDGS TDYGILQINS RWWCNDGRTP GSRNLCNIPC
SALLSSDITA SVNCAKKIVS DGNGMNAWVA WRNRCKGTDV QAWIRGCRL
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A4. Purification Strategy/Source

Sigma-Aldrich GmbH
L6876

A5. Stock Concentration/Stock Buffer

128 µg lyophilized powder

A6. Molecular Weight/Extinction Coefficient

14.3 kDa
37,970 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

Phosphate-buffered saline (PBS), pH 7.4, 0.01% Pluronic® F-127

A8. Labeling Strategy

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

A9. Labeling Procedure

1. Resuspend 128 µg of lysozyme in 43 µL of dilution buffer to obtain a ~208 µM lysozyme solution.
2. Add 25 µL of DMSO to Dye RED-NHS 2nd Generation (10 µg) to obtain a ~600 µM solution. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
3. Transfer 1 µL of the ~600 µM dye solution into a new PCR tube.
4. Add 29 µL of the ~208 µM lysozyme solution to the tube and mix well (30 µL final volume, ~3% final DMSO concentration, ~200 µM final lysozyme concentration, ~20 µM final dye concentration[1]).
5. Incubate for 20 minutes at room temperature in the dark.

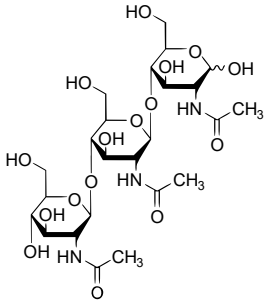
A10. Labeling Efficiency

N/A[2]

[1] The excess of protein over dye assures that a maximum of dye can react with protein before hydrolysis.
[2] No purification from unreacted dye.

B1. Ligand/Non-Fluorescent Binding Partner

Tri-N-acetyl-D-glucosamine (NAG_3)



B2. Molecule Class/Organism

Carbohydrate

B3. Sequence/Formula

$C_{24}H_{41}N_3O_{16}$

B4. Purification Strategy/Source

Sigma-Aldrich GmbH
T2144

B5. Stock Concentration/Stock Buffer

25 µg powder

B6. Molecular Weight/Extinction Coefficient

627.59 Da

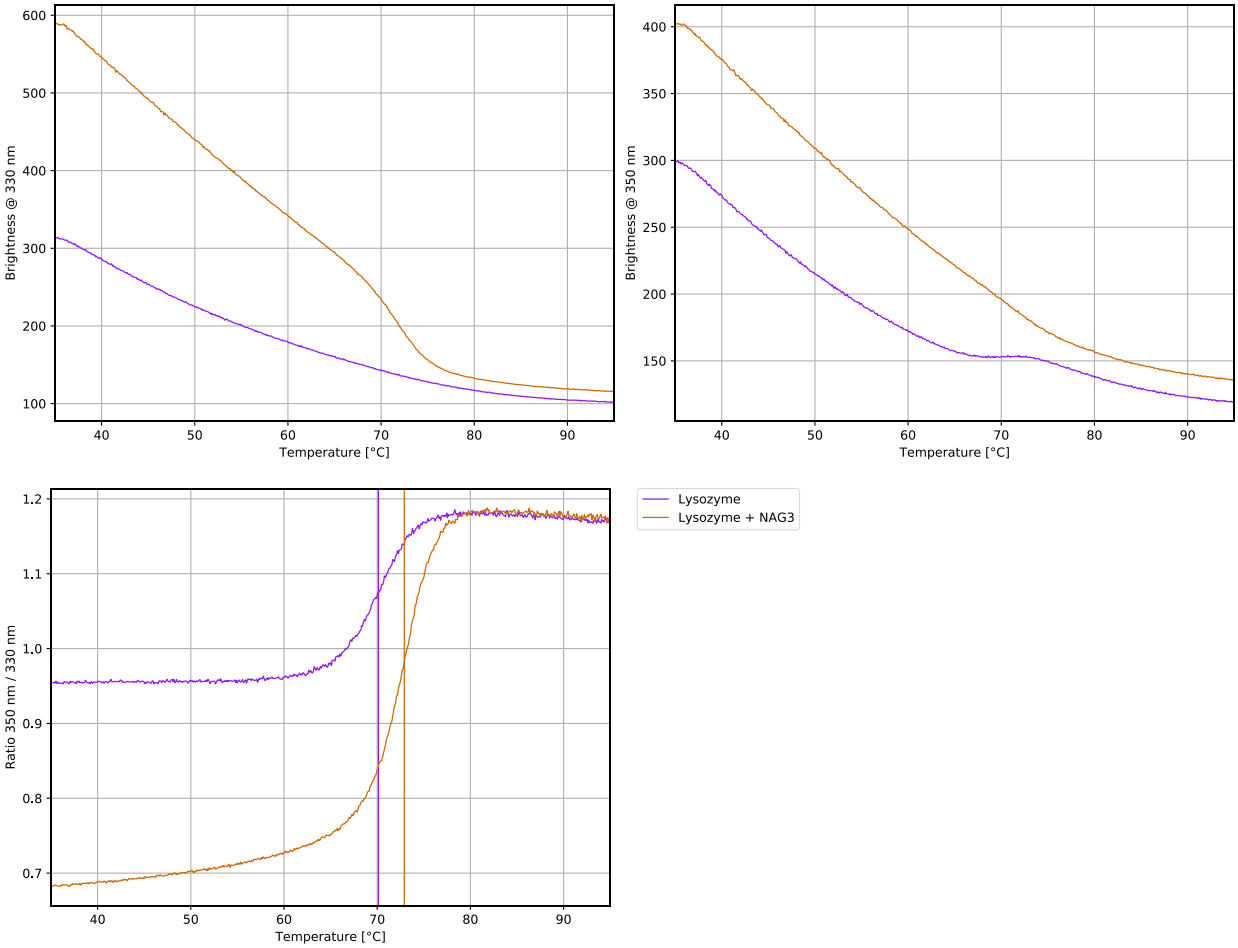
B7. Serial Dilution Preparation

1. Resuspend NAG_3 in 25 µL of dilution buffer to obtain a 1.6 mM solution.
2. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 1.6 mM NAG_3 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 1 µL of labeled lysozyme (~200 µM) with 199 µL of dilution buffer to obtain 200 µL of ~1 µM labeled lysozyme solution.
5. Add 10 µL of labeled lysozyme (~1 µM) to each tube from **16** to **1** and mix by pipetting.
6. Incubate for 5 minutes at room temperature in the dark before loading capillaries.

C. Applied Quality Checks

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

Lysozyme	6 µL of ~208 µM lysozyme + 4 µL of dilution buffer	T_i = 70.1°C
Lysozyme + NAG3	6 µL of ~208 µM lysozyme + 4 µL of 1.6 mM NAG_3	T_i = 72.9°C



D1. MST System/Capillaries

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

D2. MST Software

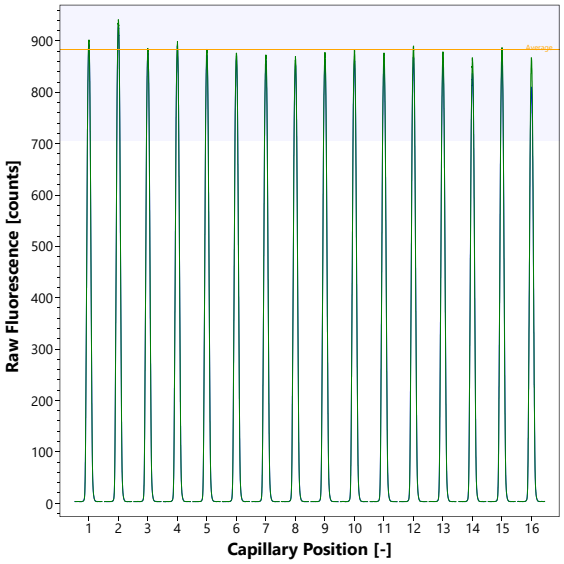
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

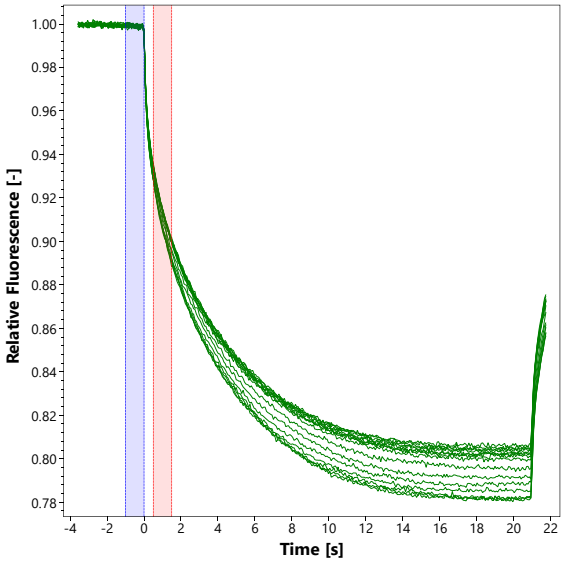
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

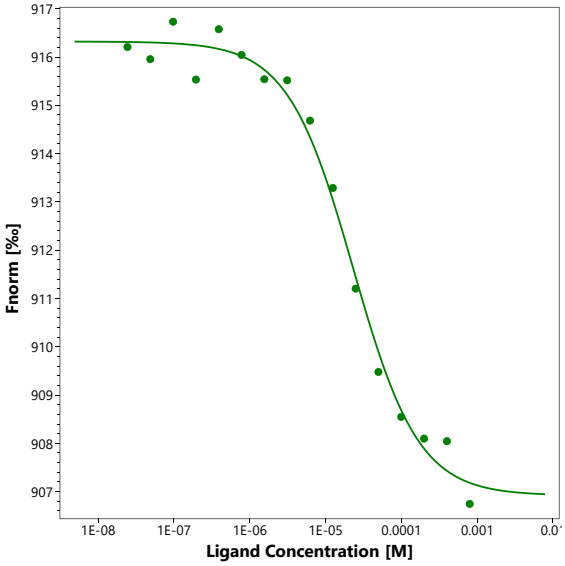
Phosphate-buffered saline (PBS), pH 7.4, 0.01% Pluronic® F-127
500 nM lysozyme (~50 nM dye) | 800 µM – 24 nM NAG_3 | 22°C | medium MST Power | 20% excitation power

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

K_d = 23 µM







D5. Reference Results/Supporting Results

K_d = 39.8 µM	NanoESI Jecklin et al., J Am Soc Mass Spectrom 19 (2008) 332–343
K_d = 39 µM	Microchip-ESI-MS Svobodova et al., Electrophoresis 31 (2010) 2680–2685

E. Contributors

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