Monolith Protocol MO-P-009

Lysozyme – NAG_3

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 interaction | carbohydrate

A1. Target/Fluorescent Molecule

Lysozyme
uniprot.org/uniprot/B8YK79

A2. Molecule Class/Organism

Glycoside hydrolase
Gallus gallus (Chicken)

A3. Sequence/Formula

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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

32 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* Labeling Buffer NHS | 1* Dye RED-NHS 2nd Generation (10 µg) | 1* B-Column

A9. Labeling Procedure

1. Resuspend 32 µg lysozyme in 112 µL of Labeling Buffer NHS to obtain a 20 µM solution.
2. Transfer 80 µL of the 20 µM lysozyme solution into a new tube.
3. 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.
4. Mix 10 µL of the 600 µM dye solution with 70 µL of Labeling Buffer NHS to obtain 80 µL of a 75 µM dye solution (~4x protein concentration).
5. Mix lysozyme and dye in a 1:1 volume ratio (160 µL final volume, ~6% final DMSO concentration).
6. Incubate for 20 minutes at room temperature in the dark.
7. 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.
8. 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.
9. Add 160 µL of the labeling reaction from step 5 to the center of the column and let sample enter the resin bed completely.
10. Add 500 µL of dilution buffer after the sample has entered and discard the flow through.
11. Place column in a new collection tube, add 400 µL of dilution buffer and collect the eluate.
12. Keep the labeled lysozyme (~4 µM) on ice in the dark.

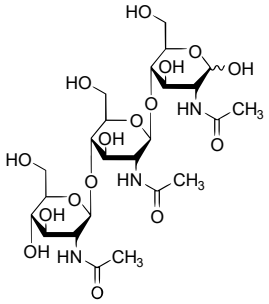
A10. Labeling Efficiency

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

Absorbance A_{280}	0.117	Protein concentration	~2.57 µM
Absorbance A_{650}	0.490	Degree-of-labeling (DOL)	~0.98

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

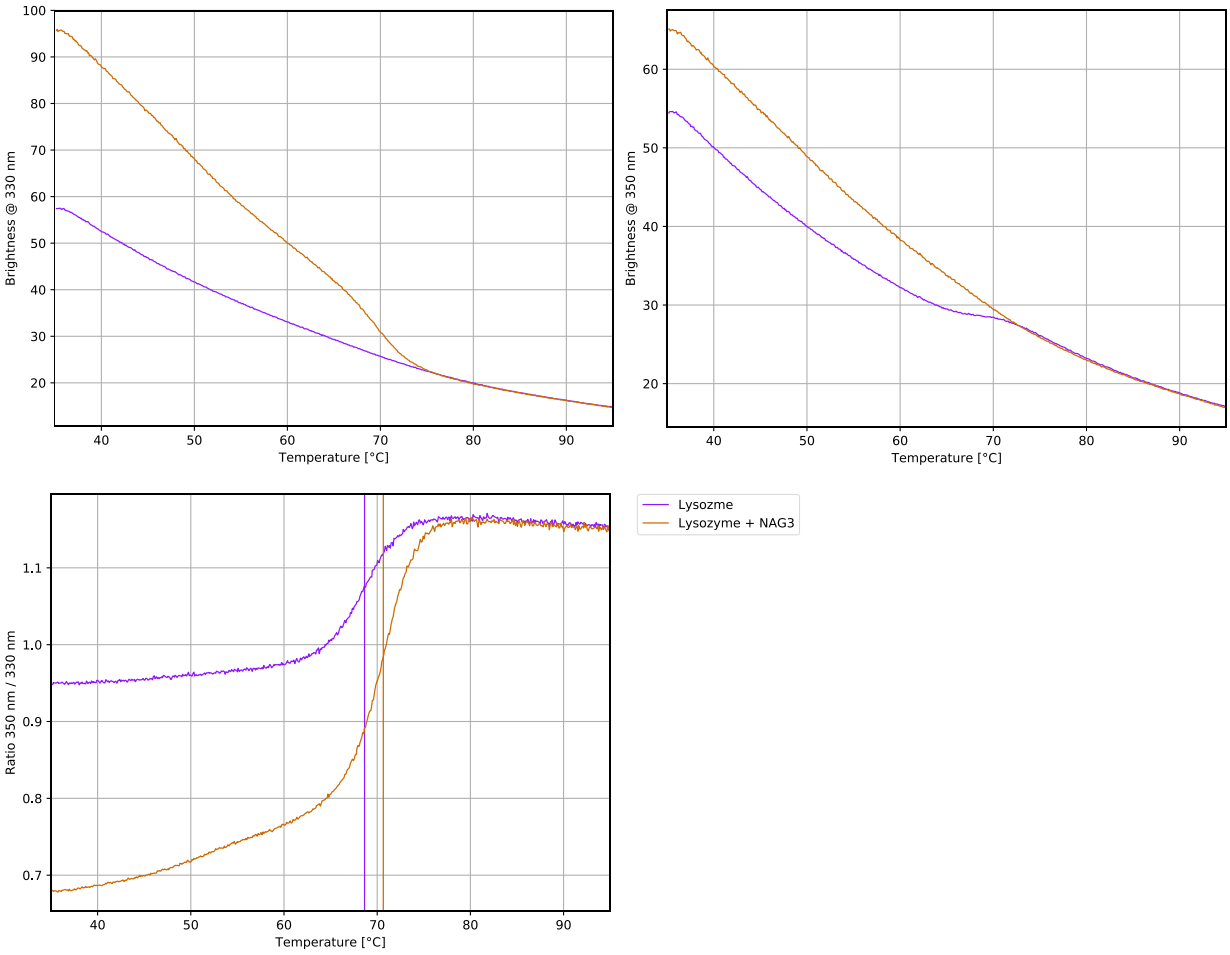
B7. Serial Dilution Preparation

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 4 µL of labeled lysozyme (~4 µM) with 196 µL of dilution buffer to obtain 200 µL of a ~100 nM lysozyme solution.
5. Add 10 µL of labeled lysozyme (~100 nM) 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	8 µL of 20 µM lysozyme + 2 µL of dilution buffer	T_i = 68.6°C
Lysozyme + NAG_3	8 µL of 20 µM lysozyme + 2 µL of 1.6 mM NAG_3	T_i = 70.7°C



D1. MST System/Capillaries

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

D2. MST Software

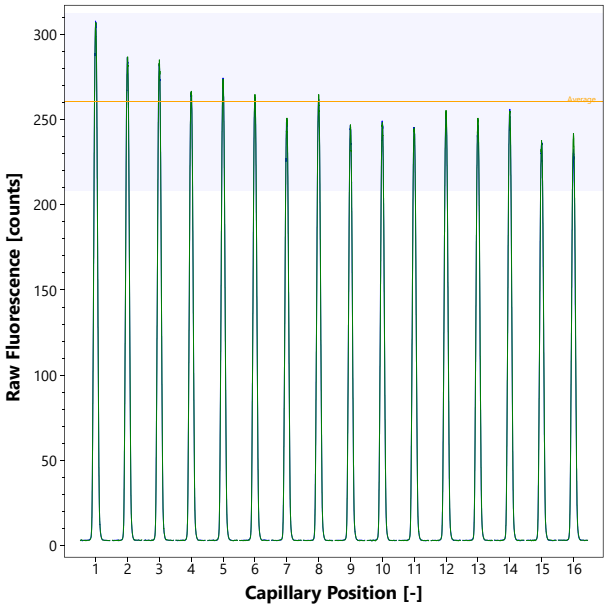
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

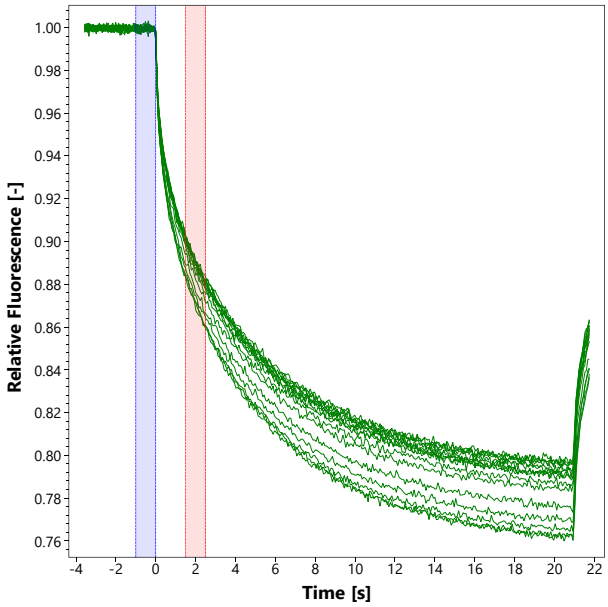
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

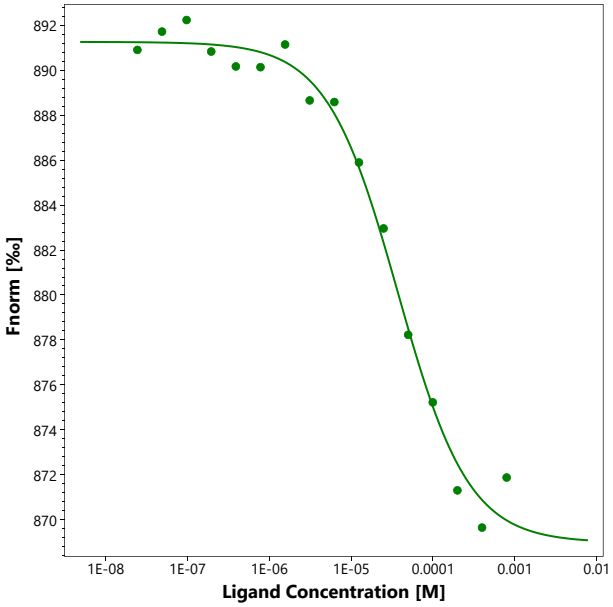
Phosphate-buffered saline (PBS), pH 7.4, 0.01% Pluronic® F-127
50 nM lysozyme | 800 µM – 24 nM NAG_3 | 22°C | medium MST Power | 20% excitation power

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

K_d = 37.3 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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