Monolith Protocol MO-P-053

# Lysozyme – $NAG_3$ (label-free)

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 | label-free

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## 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
```

## 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 $M^{-1}cm^{-1}$ ($\varepsilon_{280}$)

## A7. Dilution Buffer

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

## A8. Labeling Strategy

Trp and Tyr fluorescence

## A9. Labeling Procedure

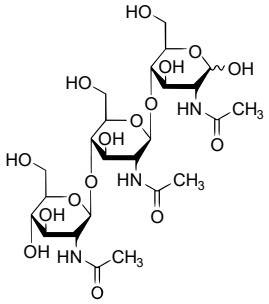
1. Resuspend lysozyme in 100 µL of dilution buffer to obtain a 20 µM solution.
2. Transfer 85 µL of this solution into a fresh tube and mix with 85 µL of dilution buffer to obtain 170 µL of a 10 µM lysozyme solution.

## A10. Labeling Efficiency

N/A

## 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

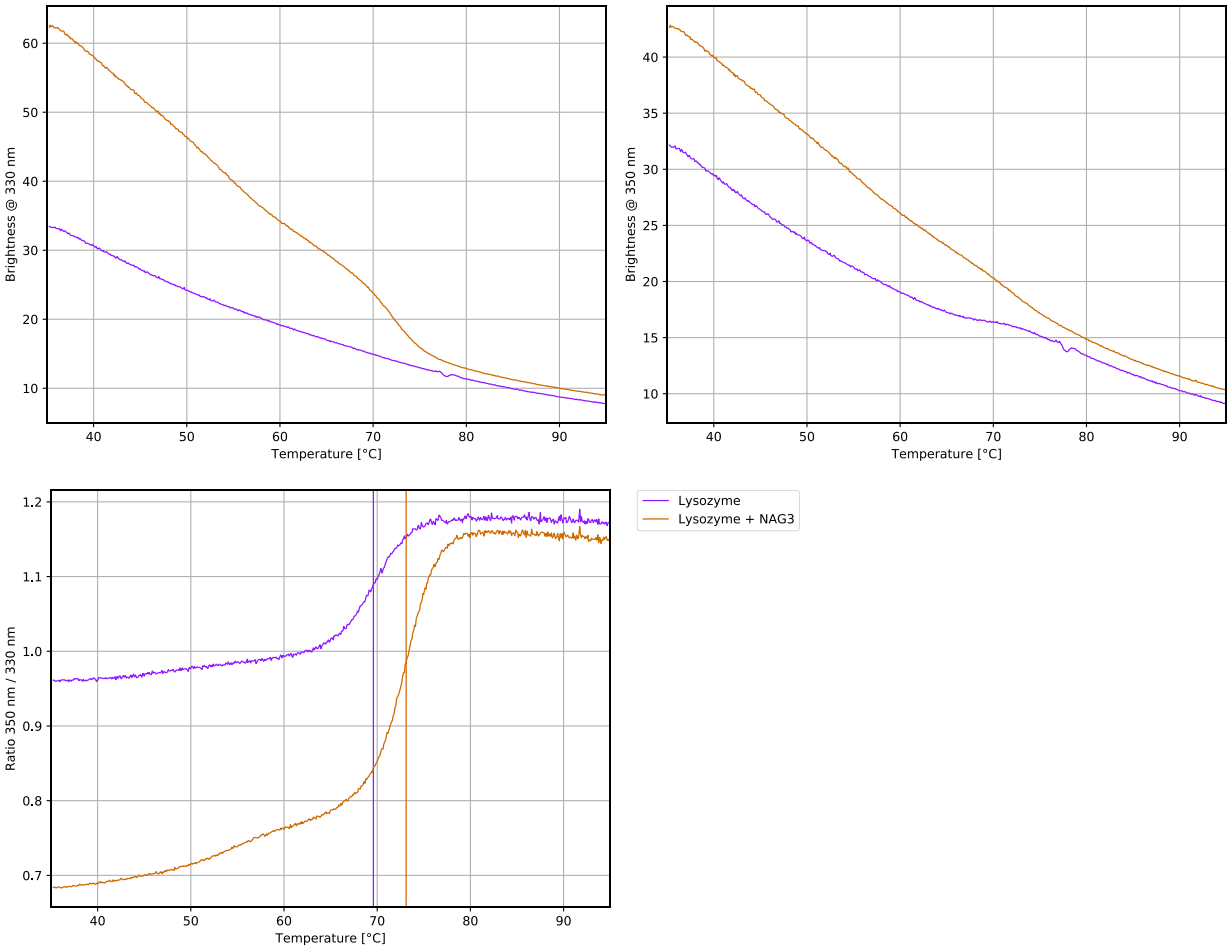
## B7. Serial Dilution Preparation

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. Add 10 µL of lysozyme (10 µM) to each tube from **16** to **1** and mix by pipetting.
5. 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 | 5 µL of 20 µM lysozyme + 5 µL of dilution buffer | $T_i$ = 69.6°C |
| Lysozyme + NAG3 | 5 µL of 20 µM lysozyme + 5 µL of 1.6 mM $NAG_3$ | $T_i$ = 73.1°C |




## D1. MST System/Capillaries

Monolith NT.LabelFree (NanoTemper Technologies GmbH)
Premium Capillaries Monolith NT.LabelFree (MO-Z025, NanoTemper Technologies GmbH)

## D2. MST Software

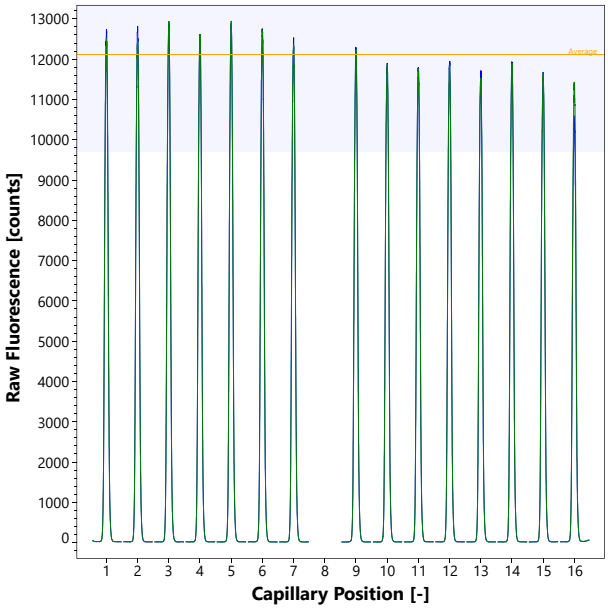
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

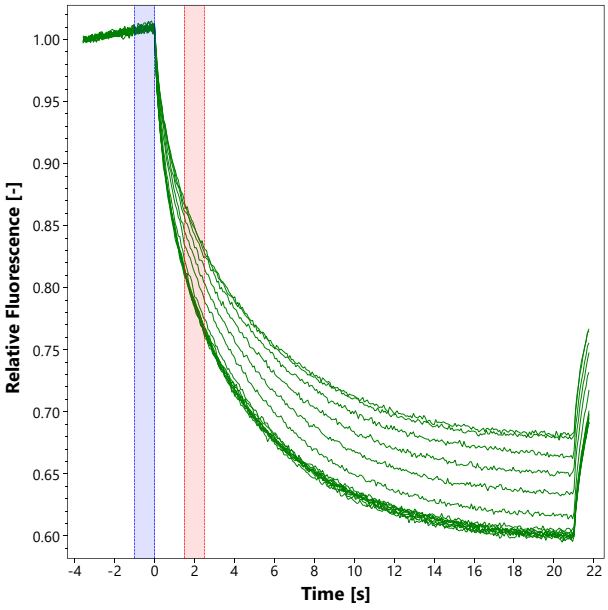
## D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

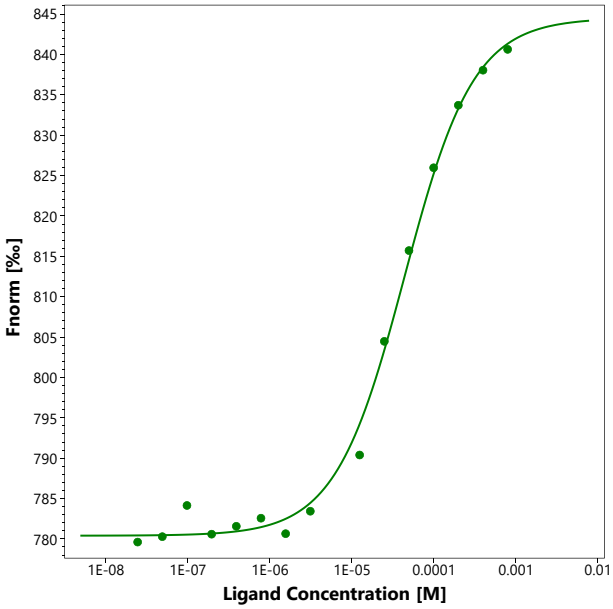
Phosphate-buffered saline (PBS), pH 7.4, 0.01% Pluronic® F-127
5 µM lysozyme | 800 µM – 24 nM $NAG_3$ | 22°C | medium MST power | 10% excitation power

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

$K_d$ = 32.9 µM



## D5. Reference Results/Supporting Results

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

## E. Contributors

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