Prometheus Protocol PR-P-001

Thermal Unfolding of Lysozyme

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.

thermal unfolding | conformational stability | colloidal stability | aggregation | T_m | T_{on} | T_{agg}

A1. Target/Fluorescent Molecule

Lysozyme
uniprot.org/uniprot/B8YK79

A2. Molecule Class/Organism

Glycoside hydrolases
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 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

50 mM Tris-HCl, pH 7.8, 150 mM NaCl, 10 mM $MgCl_2$, 0.05% TWEEN® 20

D1. nanoDSF System/Capillaries

Prometheus NT.48 (NanoTemper Technologies GmbH)
Prometheus Aggregation Detection Optics (PR-AGO, NanoTemper Technologies GmbH)
Standard Capillaries Prometheus NT.48 nanoDSF Grade (PR-C002, NanoTemper Technologies GmbH)

D2. nanoDSF Software

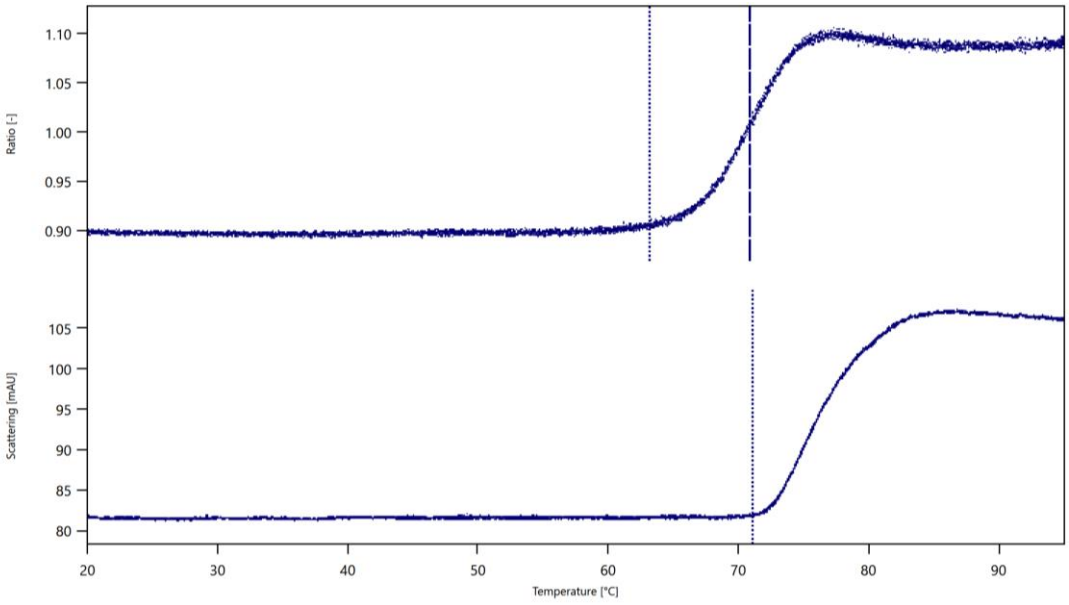
PR.ThermControl v2.1 (NanoTemper Technologies GmbH)
nanotempertech.com/prometheus-software

D3. nanoDSF Experiment

1. Resuspend lysozyme in 32 µL of dilution buffer to obtain a 1 mg/mL solution.
2. Completely fill one capillary from this solution, place it on position 1 of the capillary tray and place the magnetic lid to fix the capillary.
3. Start a new session of the *PR.ThermControl* software.
4. Go to 'Melting Scan' and prepare a run with the following settings:
    a. Only capillary 1 selected
    b. 1.0°C/min
    c. 20°C – 95°C
    d. 20% excitation power
5. Start the measurement.

D4. nanoDSF Results

T_m = 70.9°C | T_{on} = 63.2°C | T_{agg} = 71.1°C



D5. Reference Results/Supporting Results

T_m = 70.3°C	FTIR spectroscopy Venkataramani et al., J Pharm Bioallied Sci. 2 (2013) 148–153
T_m = 71.7°C	Differential Scanning Calorimetry (DSC) James et al., J Phys Chem B 116 (2012) 10182–10188

E. Contributors

Andreas Langer[1]

[1] NanoTemper Technologies GmbH, München, Germany | nanotempertech.com