Prometheus Protocol PR-P-010

Thermodynamics of Lysozyme Unfolding

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. In a thermal unfolding measurement in Prometheus, in which it is assured that the protein does not aggregate (e.g. by choosing an appropriate unfolding buffer), not only the transition temperature T_m, but also the transition enthalpy ΔH_m can be determined.

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

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A1. Target/Fluorescent Molecule

Lysozyme
uniprot.org/uniprot/B8YK79

A2. Molecule Class/Organism

Glycoside hydrolases
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

0.1 M Glycine-HCl, pH 2.5

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

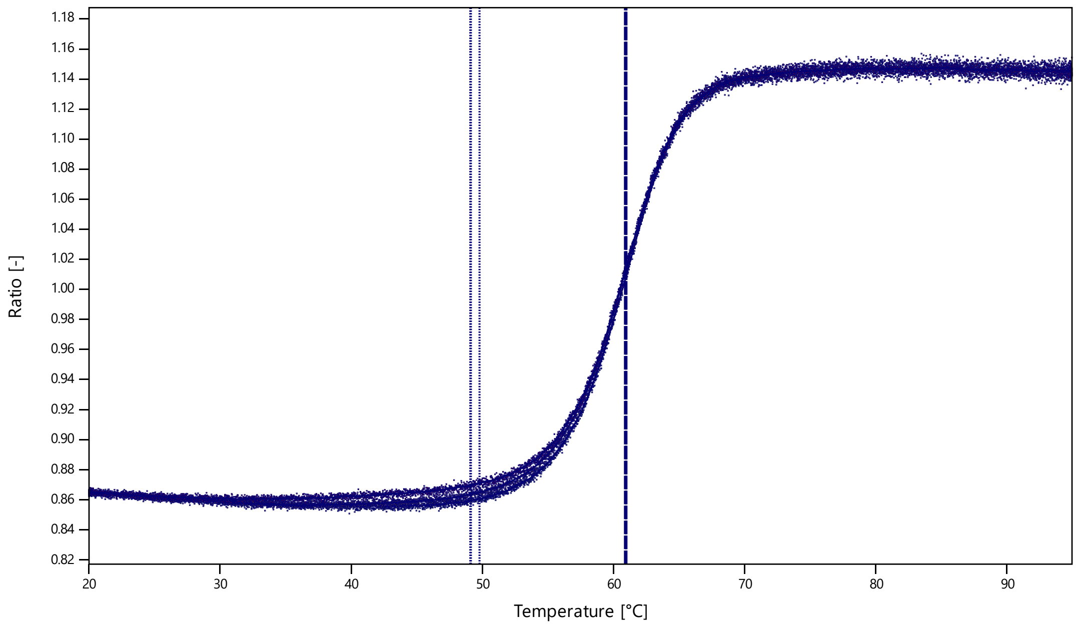
PR.ThermControl v2.1 | PR.Stability Analysis v1.1 (NanoTemper Technologies GmbH)
nanotempertech.com/prometheus-software

D3. nanoDSF Experiment

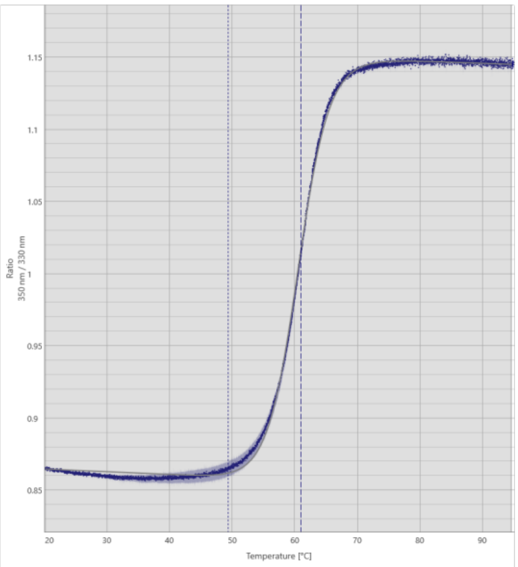
1. Resuspend lysozyme in 32 µL dilution buffer to obtain a 1 mg/mL solution.
2. Completely fill three capillaries from this solution, place it on position 1 – 3 of the capillary tray and place the magnetic lid to fix the capillaries.
3. Start a new session of the *PR.ThermControl* software.
4. Go to 'Melting Scan' and prepare a run with the following settings:
    a. Capillaries 1 – 3 selected
    b. 1.0°C/min
    c. 20°C – 95°C
    d. 5% excitation power
5. Start the measurement.
6. When the measurement is finished, open the file in PR.Stability Analysis and combine the triplicates.
7. Apply a thermodynamic fit by adding a Two-State Region of Interest (RoI) from 20 °C to 95 °C.

D4. nanoDSF Results

T_m = 60.96 ± 0.06°C | T_{on} = 49.34 ± 0.39°C



$T_m = 60.56 \pm 0.08°C$[1] | $\Delta H_m = 373.8 \pm 4.7$ kJ/mol[1]



D5. Reference Results/Supporting Results

$T_m = 56.2 – 58.7°C$ | $\Delta H_m = 377 – 439$ kJ/mol

Differential Scanning Calorimetry (DSC)
Hinz & Schwarz, J. Chem. Thermodynamics 2001, 33, 1511–1525

E. Contributors

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[1] T_m and ΔH_m were been obtained by a thermodynamic fit of the 350/330 nm ratio data in the PR.StabilityAnalysis software.
[2] NanoTemper Technologies GmbH, München, Germany | nanotempertech.com