Prometheus Protocol PR-P-011

Thermal Unfolding and Refolding of Lysozyme

Lysozyme is an enzyme with antimicrobial activity that accomplishes its function by catalyzing the hydrolysis of the peptidoglycan in the cell wall of gram-positive bacteria. Lysozyme is commercially available for a reasonable price and therefore often used as a model protein to study protein unfolding, refolding, aggregation and protein-protein interactions.

thermal unfolding | thermal refolding | unfolding reversibility | aggregation

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A1. Target/Fluorescent Molecule

Lysozyme (from chicken egg white)
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
62971

A5. Stock Concentration/Stock Buffer

10 g crystalline powder

A6. Molecular Weight/Extinction Coefficient

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

A7. Dilution Buffer

25 mM sodium phosphate, pH 2.5 | 25 mM sodium acetate, pH 5.0 | 25 mM sodium phosphate, pH 7.0

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)
Capillary Sealing Paste Prometheus Series (PR-P001, NanoTemper Technologies GmbH)
Capillary Sealing Applicators Prometheus Series (PR-P002, 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

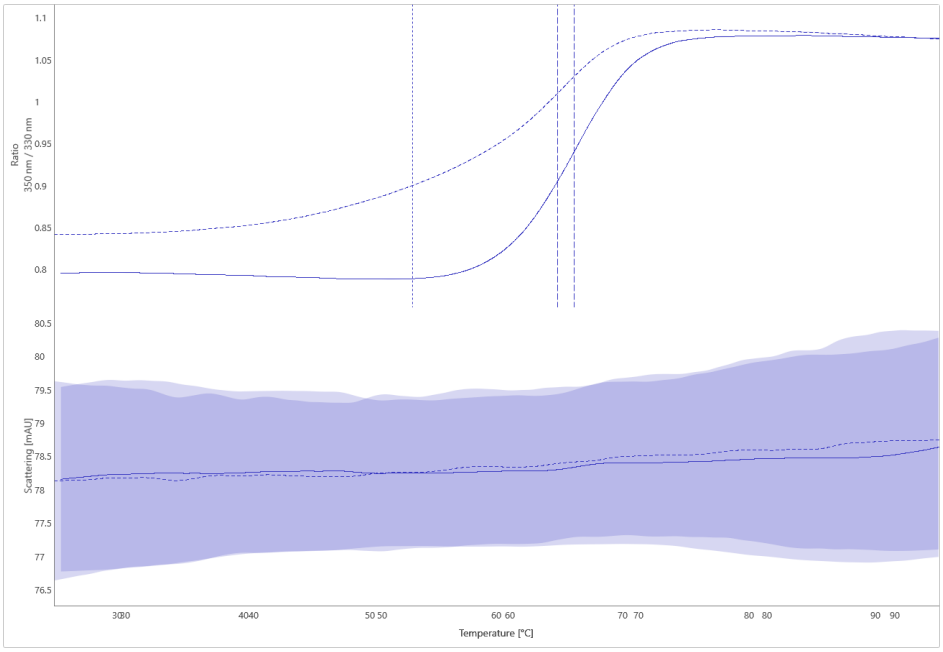
1. Prepare a 10 mg/mL solution of lysozyme in the three different dilution buffers.
2. Completely fill three capillaries from each solution, place them on position 1 – 9 of the capillary tray and place the magnetic lid to fix the capillaries.
3. Seal the capillaries using the Capillary Sealing Paste and the Capillary Sealing Applicators.
4. Start a new session of the *PR.ThermControl* software.
5. Perform a discovery scan with 10% excitation power.
6. Go to 'Melting Scan' and prepare a run with the following settings:
    a. Capillaries 1 – 9 selected
    b. 1.0°C/min
    c. 20°C – 95°C
    d. 10% excitation power
    e. Use refolding ramp
7. Start the measurement.
8. Label the samples.
9. When the measurement is finished, open the file in *PR.Stability Analysis* and combine the triplicates.

D4. nanoDSF Results

Unfolding and refolding[1] in 25 mM sodium phosphate, pH 2.5

Unfolding: IP = 65.00 ± 0.01 °C | T_{on} = 52.41 ± 0.04 °C | T_{agg} = N/A

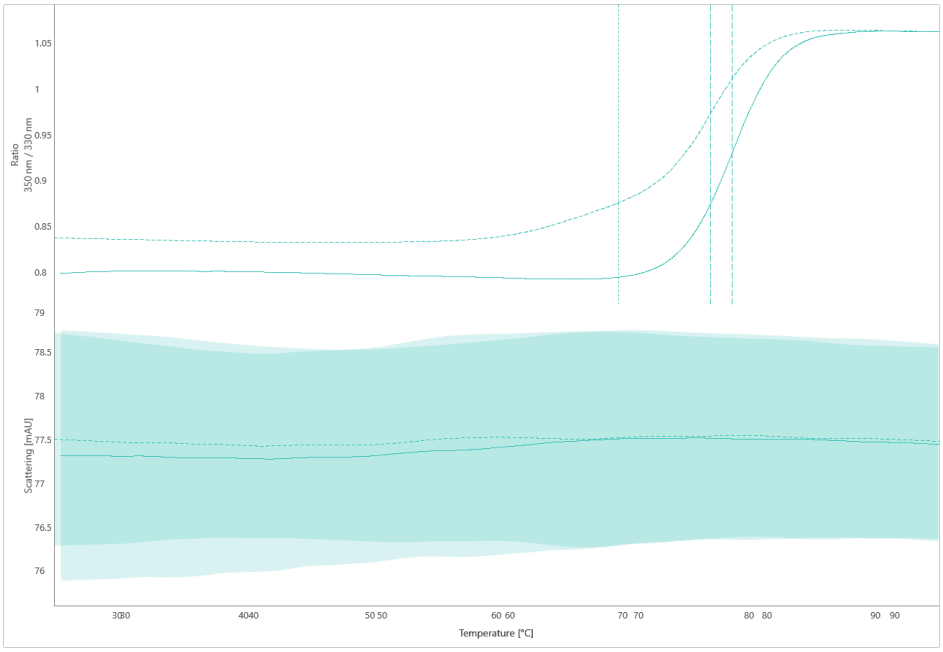
Refolding: IP = 64.88 ± 0.10 °C



Unfolding and refolding in 25 mM sodium acetate, pH 5.0

Unfolding: IP = 77.33 ± 0.04 °C | T_{on} = 68.45 ± 0.08 °C | T_{agg} = N/A

Refolding: IP = 76.94 ± 0.04 °C

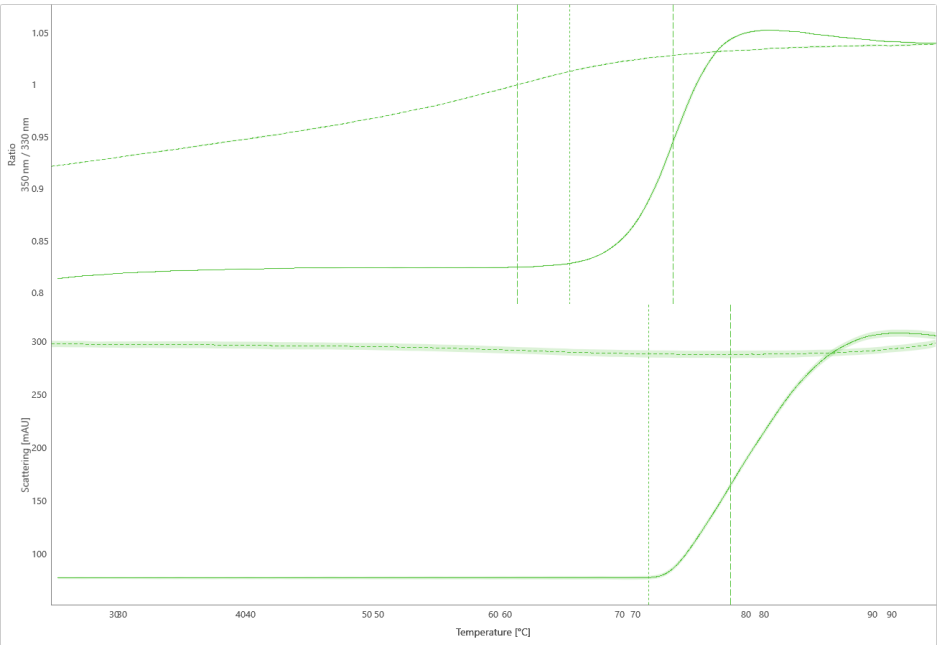


[1] The signal during cooling and refolding is the dashed line.

Unfolding and refolding in 25 mM sodium phosphate, pH 7.0

Unfolding: IP = 72.96 ± 0.10 °C | T_{on} = 64.86 ± 0.10 °C | T_{agg} = 71.01 ± 0.08 °C

Refolding: IP = 61.93 ± 0.41 °C



D5. Reference Results/Supporting Results

Reversibility of lysozyme thermal unfolding
Blumlein & McManus, BBA, 1834, 10, 2064–2070 (2013)

Stability of lysozyme solutions
Fujita et al., Bull. Chem. Soc. Jpn., 55, 1896–1900 (1982)
Klijn & Hubbuch, Int. J. Pharm., 560, 166–174 (2019)

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