Prometheus Protocol PR-P-013

Stability of Enzymes in Liquid Laundry Detergent

Many biological laundry detergents contain lipase and protease enzymes. Lipases break down fats and oils, while proteases break down protein chains. This ability makes them excellent for stain removal. Although one of the main advantages of having enzymes in washing detergent is that lower washing temperatures can be employed, the enzymes must still retain activity up to a temperature of 60°C.

thermal unfolding | aggregation | unfolding kinetics | isothermal denaturation | activation energy

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A1. Target/Fluorescent Molecule

Lipases, proteases and amylases from washing detergent

A2. Molecule Class/Organism

Lipases, proteases & amylases

A3. Sequence/Formula

N/A

A4. Purification Strategy/Source

Henkel (*Persil Tiefenrein*)
henkel.de/presse-und-medien/presseinformationen-und-pressemappen/2019-02-22-tiefenrein-gegen-hartnaeckige-flecken-913598

A5. Stock Concentration/Stock Buffer

0.4-0.8% (estimated enzyme concentration[1])
Washing detergent (15-30% anionic tenside, 5-15% non-ionic tenside, <5% soap, phosphonates)

A6. Molecular Weight/Extinction Coefficient

N/A

A7. Dilution Buffer

Phosphate buffered saline (PBS, pH 7.4)

[1] http://www1.lsbu.ac.uk/water/enztech/detergent.html

D1. nanoDSF System/Capillaries

Prometheus NT.48 (NanoTemper Technologies GmbH)
Prometheus High Temperature Upgrade (PR-HTU, NanoTemper Technologies GmbH)
High Sensitivity Capillaries Prometheus NT.48 nanoDSF Grade (PR-C006, 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

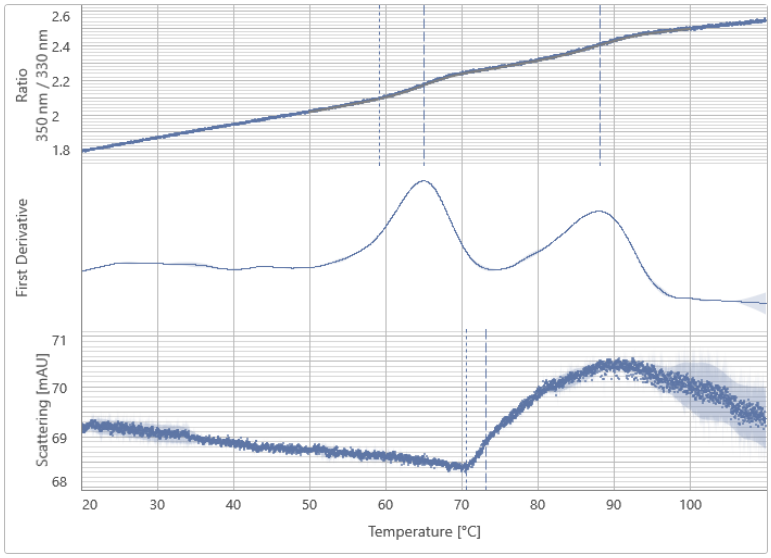
PR.ThermControl v2.1 | PR.TimeControl v1.0.2 | PR.Stability Analysis v1.1 | (NanoTemper Technologies GmbH)
nanotempertech.com/prometheus-software

D3. nanoDSF Experiment

1. Remove the top cap of a PD-10 Desalting Column (Sephadex G-25 resin, GE Healthcare) and pour off the column storage solution.
2. Remove the bottom cap and place with adapter in a 15 mL tube.
3. Equilibrate and wash the column by adding 9 mL dilution buffer (flow through by gravity flow).
4. Add 200 µL of washing detergent to the center of the column and let sample enter the bed completely.
5. Add 400 µL dilution buffer after the sample has entered and discard the flow through.
6. Place column in a new 15 mL collection tube, add 500 µL of dilution buffer and collect the eluate.
7. Start a new session of the *PR.ThermControl* software.
8. Completely fill two capillaries from the eluate of step 6, place them on positions 1 and 2 of the capillary tray and place the magnetic lid to fix the capillaries.
9. Seal the capillary with capillary sealing paste, using the Capillary Sealing Applicators.
10. Go to 'Melting Scan' and prepare a run with the following settings:
    a. Only capillaries 1 and 2 selected
    b. 1.0°C/min
    c. 20°C – 110°C
    d. 10% excitation power
11. Start the measurement.
12. After the measurement is finished, start a new session of the *PR.TimeControl* software.
13. Change the Thermostat Set Point to 58°C by using the touch display on the instrument and wait for the system to reach this temperature.
14. Go to 'Measurement Scan' and prepare a run with the following settings:
    a. Only capillaries 1 and 2 selected
    b. Isothermal
    c. 58°C
    d. 20 min
    e. 10% excitation power
15. Load two capillaries from the eluate of step 6 and place them on positions 1 and 2 of the Prometheus capillary tray.
16. Place the magnetic lid to fix the capillary and start the measurement.
17. After the measurement has finished, change the Thermostat Set Point at the instrument display to 60°C and repeat steps 14 – 17 for temperatures of 60°C, 62°C, 64°C, 66°C and 68°C.

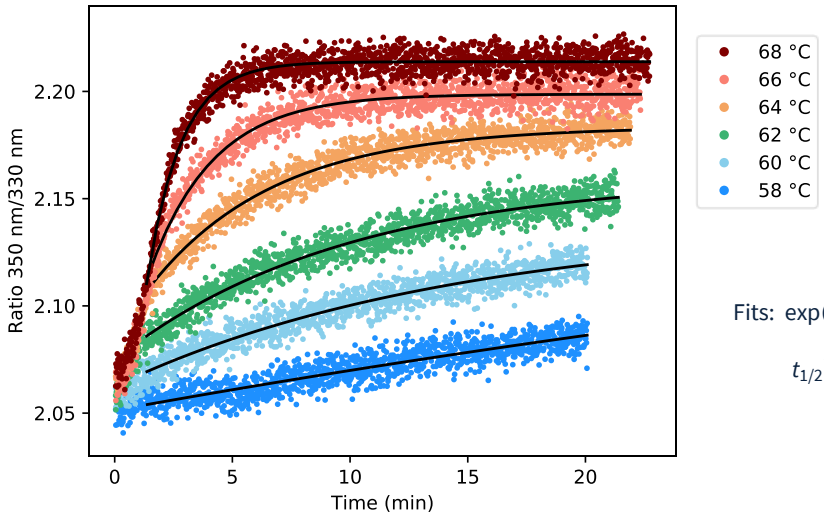
D4. nanoDSF Results

T_{on} = 59.2°C | T_{agg} = 73.2°C | $T_{m,1}$ = 65.5°C | $T_{m,2}$ = 89.2°C



T (°C)	68	66	64	62	60	58
Measurement duration (min)	20	20	20	20	20	20
k_{uf} (10^{-3} s^{-1})	11	5.7	3.0	1.7	0.95	0.44
Half-life $t_{1/2}{}^{uf}$ (min)	1.0	2.0	3.8	6.8	12.1	26.1

Kinetic data from PR.TimeControl (data points) and mono-exponential fits[2] (solid lines):



Fits: $\exp(-k_{uf} \cdot t)$

$$t_{1/2}{}^{uf} = \frac{0.69}{k_{uf}}$$

[2] Exponential fits of the data were performed using CurveExpert Basic software.

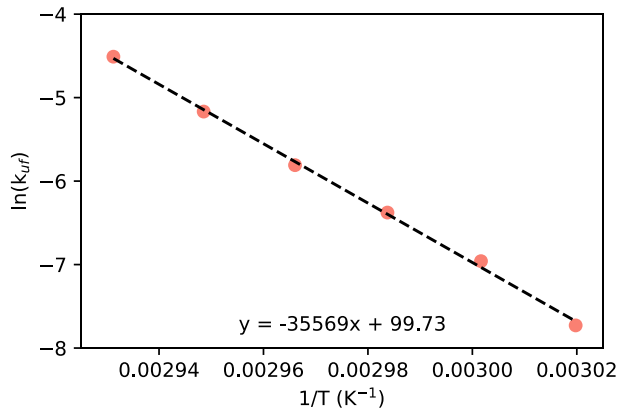
The temperature dependent (irreversible) unfolding rate is described by the Arrhenius equation:

$$k_{\mathrm{uf}} = A \cdot \exp\left(-\frac{E_{\mathrm{a}}}{RT}\right) \tag{1}$$

where A is the frequency factor (s^{-1}) and E_a is the activation energy ($\mathrm{kJ \cdot mol^{-1}}$). It follows:

$$\ln(k_{\mathrm{uf}}) = \ln(A) - \frac{E_a}{RT} \tag{2}$$

Plotting of $\ln(k_{\mathrm{uf}})$ vs. $1/T$ yields:



From linear fitting, one obtains:

$$\frac{E_{\mathrm{a}}}{R} = 35{,}569\ \mathrm{K} \quad \rightarrow \quad E_{\mathrm{a}} = 8.314\,\frac{\mathrm{J}}{\mathrm{K \cdot mol}} \cdot 35{,}569\ \mathrm{K} \ = \ 296\ \mathrm{kJ\ mol^{-1}}$$

D5. Reference Results/Supporting Results

T_m^{lipase} = 69.7°C | $T_m^{protease}$ = 73.6°C | $T_m^{amylase}$ = 96.6°C	Differential Scanning Calorimetry (DSC) Lund et al. J Surfact Deterg (2012) 15:9–21

E. Contributors

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