Prometheus Protocol PR-P-005

Temperature-Dependent Aggregation of Streptavidin

Streptavidin (SA) is a homo-tetramer that has an extraordinarily high affinity for biotin (also known as vitamin B7). It is used extensively in molecular biology and bio-nanotechnology due to the streptavidin-biotin complex's resistance to organic solvents, denaturants (e.g. guanidinium chloride), detergents (e.g. SDS, Triton), proteolytic enzymes, and extremes of temperature and pH. Under certain conditions, however, it has been shown to form higher-order oligomeric forms.

thermal unfolding | isothermal | aggregation | resolubilization

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A1. Target/Fluorescent Molecule

Streptavidin (SA)
uniprot.org/uniprot/P22629

A2. Molecular Class/Organism

Extracellular proteins
Streptomyces avidinii

A3. Sequence/Formula

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DPSKESKAQA AVAEAGITGT WYNQLGSTFI VTANPDGSLT GTYESAVGNA ESRYVLTGRY DSTPATDGSG TALGWTVAWK
NNYRNAHSAT TWSGQYVGGA EARINTQWLL TSGTTAANAW KSTLVGHDTF TKVKPSAASI DAAKKAGVNN GNPLDAVQQ
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A4. Purification Strategy/Source

Recombinant, produced in *E. coli*.
ProSpec-Tany TechnoGene Ltd.
PRO-791

A5. Stock Concentration/Stock Buffer

1 mg/mL | 19 µM
10 mM potassium phosphate buffer, pH 6.5

A6. Molecular Weight/Extinction Coefficient

52.8 kDa
167,000 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

10 mM potassium phosphate buffer, pH 6.5

D1. nanoDSF System/Capillaries

Prometheus NT.48 (NanoTemper Technologies GmbH)
Prometheus Aggregation Detection Optics (PR-AGO, 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 (NanoTemper Technologies GmbH)
nanotempertech.com/prometheus-software

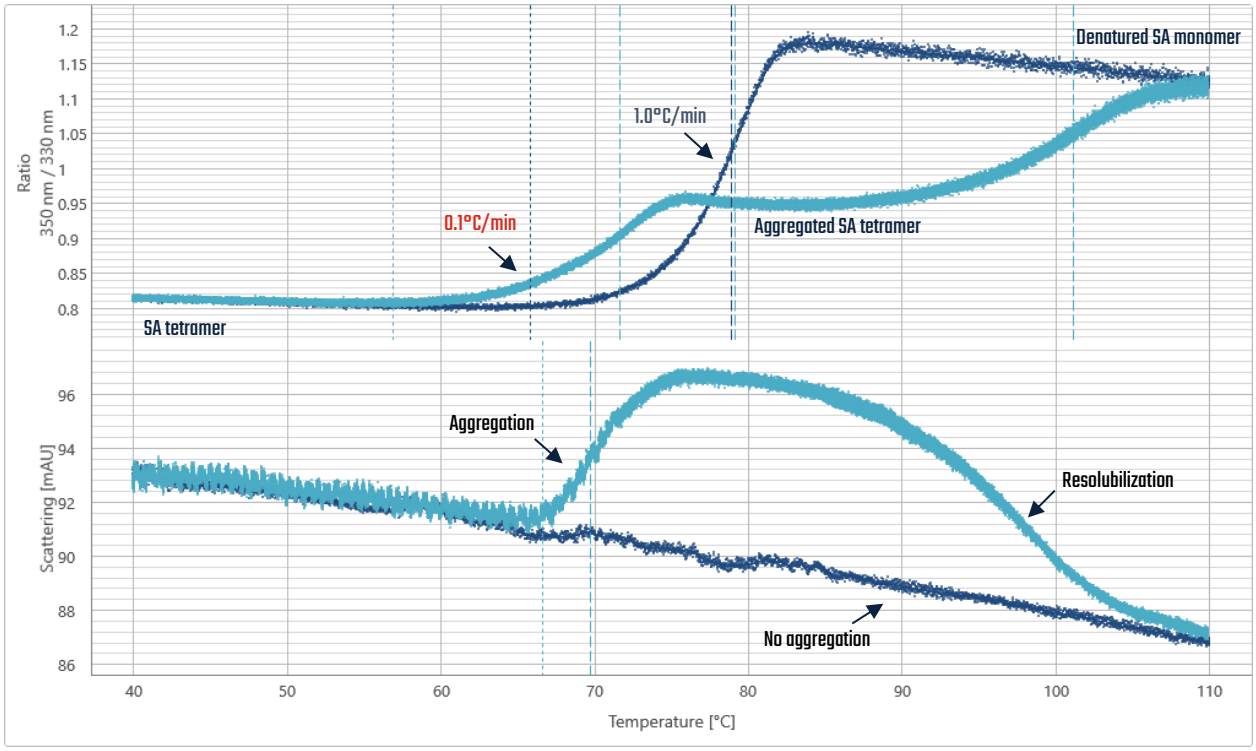
D3. nanoDSF Experiment

1. Start a new session of the *PR.ThermControl* software.
2. Load three capillaries from a 1 mg/mL SA solution and place them on positions 1 – 3 of the Prometheus capillary tray.
3. Place the magnetic lid to fix the capillaries.
4. Seal the capillaries with Capillary Sealing Paste, using the Capillary Sealing Applicators. Repeat sealing after 5 minutes.
5. Go to 'Melting Scan' and prepare a run with the following settings:
    a. Only capillaries 1 – 3 selected
    b. 0.1°C/min
    c. 40°C – 110°C
    d. 10% excitation power
6. Start the measurement (duration: ~12 hours).
7. After the measurement is finished, repeat steps 1 – 6 with a heating-rate of 1.0°C/min (step 5b).
8. Next, start a new session of the *PR.TimeControl* software.
9. Change the Thermostat Set Point to 50°C by using the touch display on the instrument and wait for the system to reach this temperature.
10. Go to 'Measurement Scan' and prepare a run with the following settings:
    a. Only capillaries 1 – 3 selected
    b. Incremental Cycle
    c. 50°C (First Temperature)
    d. 30 min (First Duration)
    e. 5°C (Temperature Increase)
    f. 60 min (Second Duration)
    g. 12 Measurement Cycles
    h. 10% excitation power
11. Load three capillaries from the 1 mg/mL SA solution and place them on positions 1 – 3 of the Prometheus capillary tray.
12. Place the magnetic lid to fix the capillaries.
13. Seal the capillaries with capillary sealing paste, using the Capillary Sealing Applicators. Repeat sealing after 5 minutes.
14. Start the measurement (duration: ~20 hours).

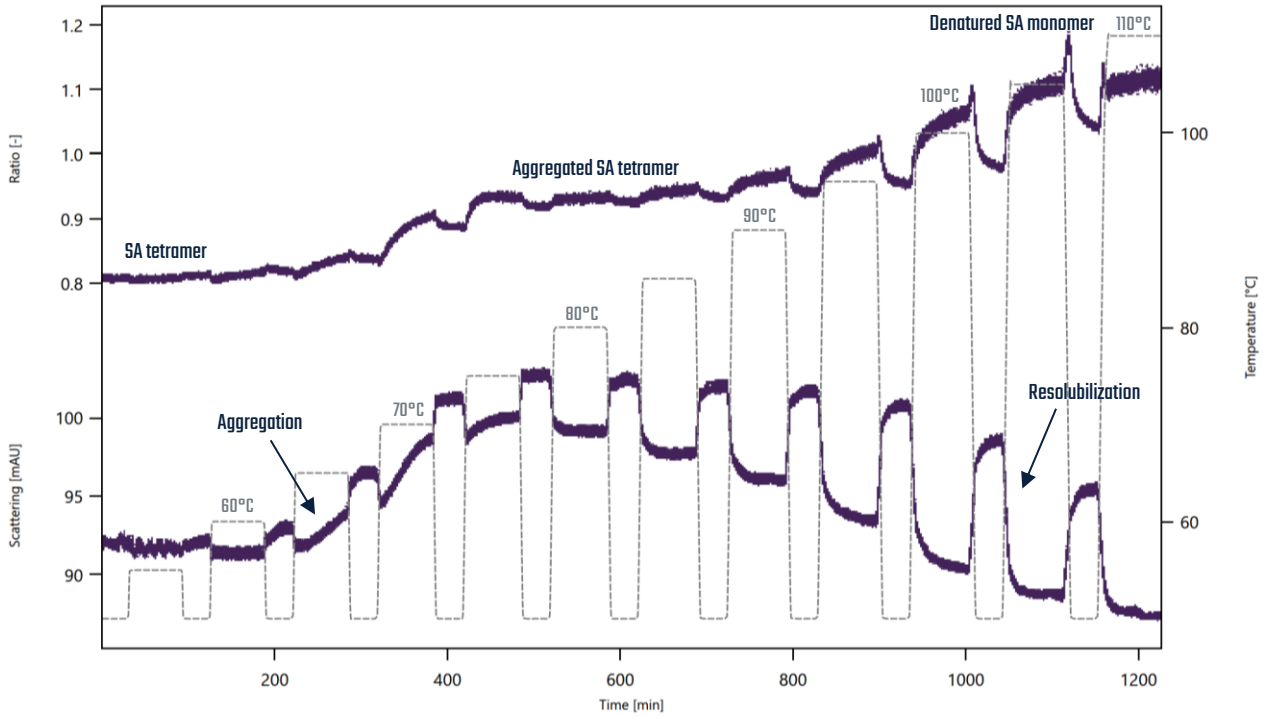
D4. nanoDSF Results

Heating-rate of 0.1°C/min: T_{on} = 56.8°C | T_{agg} = 66.6°C | T_{m1} = 71.6°C | T_{m2} = 101.2°C

Heating-rate of 1.0°C/min: T_{on} = 65.9°C | T_m = 79.0°C | **no** aggregation



Onset of aggregation: ~65°C | Onset of aggregate resolubilization: ~85°C



D5. Reference Results/Supporting Results

Aggregation of the native SA tetramer into higher-order oligomeric forms under certain conditions, though only relatively small amounts at room temperature.
Bayer et al., Biochem J 259 (1989) 369-376

Complete conversion to of tetrameric SA to monomeric SA above 80°C and heating times of 3 min, aggregation at temperatures between 65°C and 75°C and heating times of 45 min.
Waner et al., Biophys J 87 (2004) 2701–2713

Aggregation at a lower temperature range, soluble denatured molecules at high temperatures.
Leppiniemi et al., Protein Science 22 (2013) 980—994

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