

Prometheus Protocol PR-P-006

Isothermal Denaturation of Biotin-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. Biotin binding increases the midpoint of temperature T_m of thermally induced denaturation from around 75°C to over 110°C at full biotin saturation. This thermostability is the highest reported for proteins coming from either mesophilic or thermophilic organisms.

[thermal unfolding](#) | [unfolding kinetics](#) | [isothermal](#)

A1. Target/Fluorescent Molecule

Streptavidin (SA)

uniprot.org/uniprot/P22629

A2. Molecular Class/Organism

Extracellular proteins

Streptomyces avidinii

A3. Sequence/Formula

DPSKESKAQA AVAEAGITGT WYNQLGSTFI VTANPDGSLT GTYESAVGNA ESRVLTGRY DSTPATDGSG TALGWTVAWK
NNYRNAHSAT TWSGQYVGG AEARINTQWLL TSGTTAANAW KSTLVGHDTF TKVKPSAASI DAAKAGVNN GNPLDAVQQ

A4. Purification Strategy/Source

Recombinant, produced in *E. coli*.

ProSpec-Tany TechnoGene Ltd.

[PR0-791](#)

A5. Stock Concentration/Stock Buffer

1 mg/mL | 19 μ M

10 mM potassium phosphate buffer, pH 6.5

A6. Molecular Weight/Extinction Coefficient

52.8 kDa

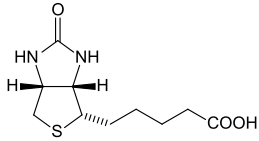
167,000 $M^{-1}cm^{-1}$ (ϵ_{280})

A7. Dilution Buffer

10 mM potassium phosphate buffer, pH 6.5

B1. Ligand/Non-Fluorescent Binding Partner

Biotin



B2. Molecule Class/Organism

Water-soluble B vitamin

B3. Sequence/Formula

$C_{10}H_{16}N_2O_3S$

B4. Purification Strategy/Source

Sigma Aldrich GmbH

[B4501](#)

B5. Stock Concentration/Stock Buffer

500 mg

Powdered

B6. Molecular Weight/Extinction Coefficient

244.31 Da

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.TimeControl v1.0.2 (NanoTemper Technologies GmbH)

nanotempertech.com/prometheus-software

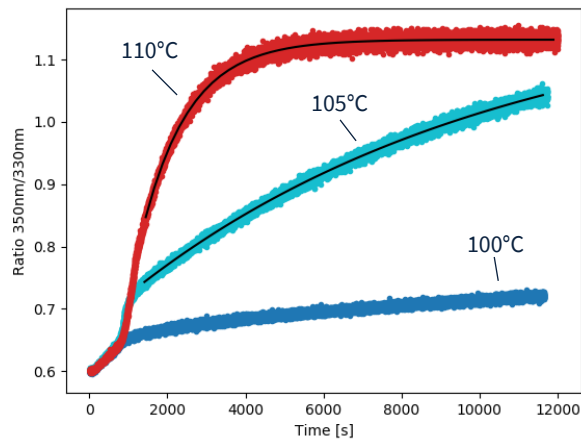
D3. nanoDSF Experiment

1. Prepare a 500 mM biotin stock solution by dissolving 100 mg biotin in 818 μL ammonium hydroxide.
2. Prepare a 1 mM biotin solution by mixing 2 μM of the 500 mM biotin stock with 998 μL of dilution buffer.
3. Add 36 μL of a 19 μM (1 mg/mL) streptavidin solution to 4 μL of the 1 mM biotin solution to obtain 40 μL of a 17 μM streptavidin, 100 μM biotin solution ($> 5\times$ streptavidin concentration)¹.
4. Start a new session of the *PR.TimeControl* software.
5. Go to 'Measurement Scan' and prepare a run with the following settings:
 - a. Only capillary 1 selected
 - b. Incremental cycle
 - c. 20°C (first temperature)
 - d. 1 min (first duration)
 - e. 90°C (temperature increase)
 - f. 180 min (second duration)
 - g. 1 measurement cycle
 - h. 10% excitation power
6. Load one capillary from the biotin-streptavidin solution of step 3 and place it on position 1 of the Prometheus capillary tray.
7. Place the magnetic lid to fix the capillary.
8. Seal the capillary with capillary sealing paste, using the Capillary Sealing Applicators. Repeat sealing after 5 minutes and one more time after another 5 minutes (triple sealing).
9. Start the measurement (duration: ~3.5 hours).
10. Repeat steps 5 – 9 with a temperature increase of 85°C (step 5e, final temperature: 105°C).
11. Repeat steps 5 – 9 with a temperature increase of 80°C (step 5e, final temperature: 100°C).

¹ As streptavidin is a tetrameric protein and each streptavidin monomer can bind one biotin molecule, a streptavidin protein can maximally bind four biotins.

D4. nanoDSF Results

T (°C)	110	105	100
Measurement duration (min)	180	180	180
$k_{\text{den}} (10^{-4} \text{ s}^{-1})$	8.3	1.1	n.a. ²
$t_{1/2}^{\text{den}} (\text{min})$	14	105	n.a. ²



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

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

D5. Reference Results/Supporting Results

$T_m = 112^\circ\text{C}$ Differential Scanning Calorimetry (DSC)
[González et al., J Biol Chem 272 \(1997\) 11288-11294](#)

E. Contributors

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² At 100°C, the streptavidin-biotin complex is still so stable that no significant amount has denatured after 3 hours.

³ NanoTemper Technologies GmbH, München, Germany | nonotempertech.com