Monolith Protocol MO-P-041

Streptavidin – d-Desthiobiotin

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. d-Desthiobiotin is a reversibly binding and stable analog of biotin that is used in affinity chromatography and protein chromatography.

protein – small molecule interaction | streptavidin

A1. Target/Fluorescent Molecule

Streptavidin (SA)
uniprot.org/uniprot/P22629

A2. Molecule 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

20 mM HEPES, pH 7.4, 150 mM NaCl, 0.005% TWEEN® 20

A8. Labeling Strategy

Monolith Protein Labeling Kit RED – NHS (MO-L001, NanoTemper Technologies GmbH)
1* 10 µg RED-NHS dye | 1* B-Column

A9. Labeling Procedure

1. Mix 52.6 µL of 19 µM SA with 47.4 µL of a 50 mM phosphate, 150 mM NaCl, **pH 7.0** buffer[1] to obtain 100 µL of a 10 µM SA solution.
2. Add 17.6 µL of DMSO to 10 µg RED-NHS dye to obtain a ~800 µM solution. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
3. Mix 10 µL of the 800 µM dye solution with 90 µL of 50 mM phosphate, 150 mM NaCl, pH 7.0 buffer to obtain 100 µL of a 80 µM dye solution (8x protein concentration / 2x monomer concentration).
4. Mix streptavidin and dye in a 1:1 volume ratio (200 µL final volume, 5% final DMSO concentration).
5. Incubate for **4 hours** at room temperature in the dark.
6. In the meantime, remove the top cap of the B-Column and pour off the storage solution. Remove the bottom cap and place with adapter in a 15 mL tube.
7. Fill the column with dilution buffer and allow it to enter the packed resin bed completely by gravity flow. Discard the flow through collected. Repeat this step 3 more times.
8. Add 200 µL of the labeling reaction from step 4 to the center of the column and let sample enter the bed completely.
9. Add 400 µL of dilution buffer after the sample has entered and discard the flow through.
10. Place column in a new collection tube, add 500 µL of dilution buffer and collect the eluate.
11. Keep the labeled SA (~2 µM) on ice in the dark.

A10. Labeling Efficiency

Measurement of protein concentration and degree of labeling (DOL) using a NanoDrop™:
nanotempertech.com/dol-calculator

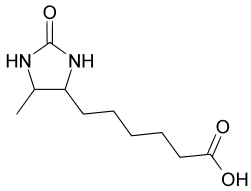
Absorbance A_{280}	0.401	Protein concentration	2.10 µM
Absorbance A_{650}	1.677	Degree-of-labeling (DOL)	3.19

[1] Dyes containing N-hydroxysuccinimide (NHS) esters are widely used to label proteins at primary amino groups (-NH_2), which exist in the side chain of **lysine residues** and at the **N-terminus** of each protein. With large proteins, labeling of lysine residues does usually not harm protein function or binding properties. In the case of streptavidin, however, the random labeling of lysine residues has been found to interfere with biotin binding (Jacobson, 2017).

The preferential labeling of the N-terminal α-amino group can be achieved by using a reaction pH that is lower than the typical range used for reaction by NHS-ester reagents (pH 8 – 8.5). Since the pKa of the α-amino group (pKa = 8.9) is considerably lower than the pKa of the ε-amino group of lysine (pKa = 10.5), this ensures that the lysine amines are very rarely in the unprotonated state that allows them to react.

B1. Ligand/Non-Fluorescent Binding Partner

d-Desthiobiotin



B2. Molecule Class/Organism

Biotin analog

B3. Sequence/Formula

$C_{10}H_{18}N_2O_3$

B4. Purification Strategy/Source

Sigma Aldrich GmbH
D1411

B5. Stock Concentration/Stock Buffer

500 mg
Powdered

B6. Molecular Weight/Extinction Coefficient

214.26 Da

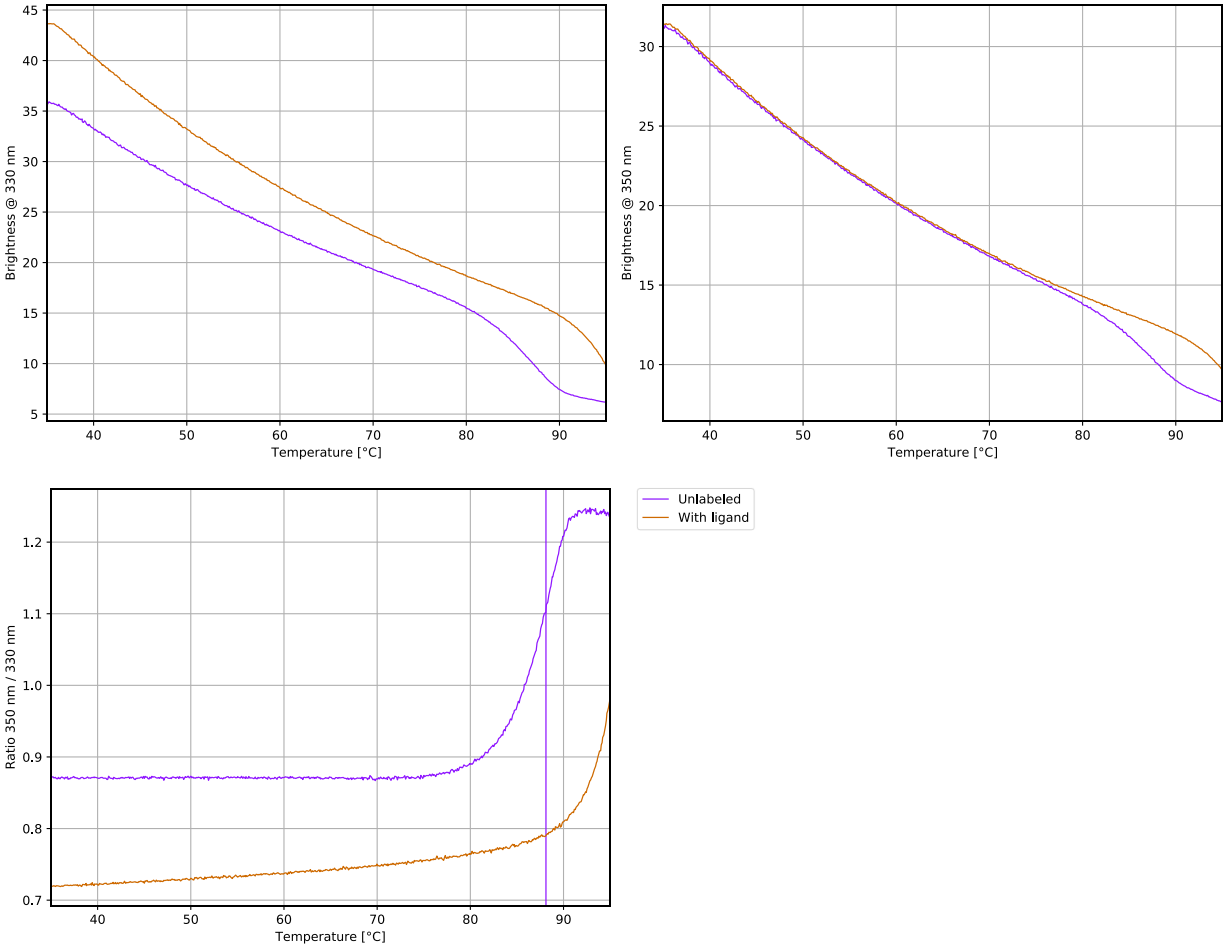
B7. Serial Dilution Preparation

1. Dissolve 21.4 mg d-Desthiobiotin in 1 mL of DMSO to obtain a 100 mM solution.
2. Mix 2 µL of 100 mM d-Desthiobiotin with 38 µL of DMSO to obtain 40 µL of a 5 mM solution.
3. Mix 2 µL of 5 mM d-Desthiobiotin with 98 µL of DMSO to obtain 100 µL of a 100 µM solution.
4. Mix 2 µL of 100 µM d-Desthiobiotin with 198 µL of dilution buffer to obtain 200 µL of a 1 µM solution.
5. Mix 2 µL of DMSO with 198 µL of dilution buffer to obtain a 1% DMSO solution.
6. Prepare a PCR-rack with 16 PCR tubes. Transfer 50 µL of the 1 µM d-Desthiobiotin solution into tube **1**. Then, transfer 50 µL of the 1% DMSO solution into tubes **2** to **16**.
7. Prepare a 1:1 serial dilution by transferring 50 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 50 µL from tube **16** to get an equal volume of 50 µL for all samples.
8. Mix 2 µL of the ~2 µM SA solution with 198 µL of dilution buffer to obtain 200 µL of a ~20 nM SA solution.
9. Mix 5 µL of the ~20 nM SA solution with 995 µL of dilution buffer to obtain 1 mL of a ~100 pM SA solution.
10. Add 50 µL of labeled SA (~100 pM) to each tube from **16** to **1** and mix by pipetting.
11. Incubate for 15 minutes at room temperature in the dark before loading capillaries.

C. Applied Quality Checks

Validation of structural integrity and functionality of SA with Tycho NT.6:
nanotempertech.com/tycho

Unlabeled	5 µL of 2 µM SA + 5 µL of dilution buffer	T_i = 88.1°C
With ligand	5 µL of 2 µM SA + 5 µL of 10 µM d-Desthiobiotin	T_i > 95°C[2]



D1. MST System/Capillaries

Monolith NT.115^{PICO} Red (NanoTemper Technologies GmbH)
Premium Capillaries Monolith NT.115 (MO-K025, NanoTemper Technologies GmbH)

D2. MST Software

MO.Control v1.6 | MO.Affinity Analysis v2.3 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

[2] Biotin has been shown to shift the T_m of streptavidin from 75°C to 112°C (González, 1997).

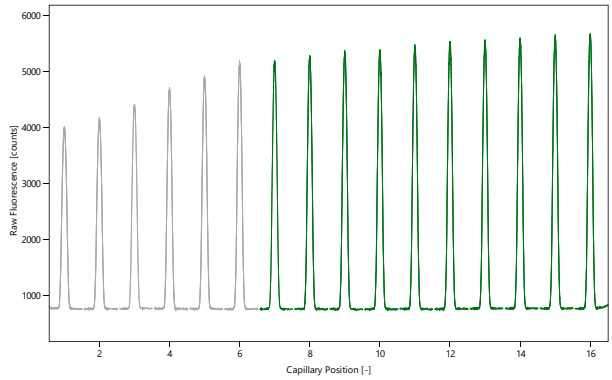
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

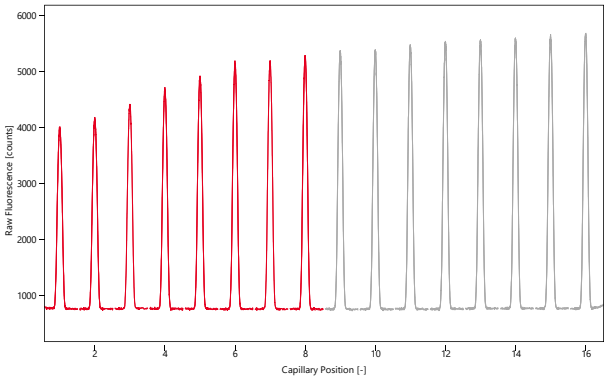
20 mM HEPES, pH 7.4, 150 mM NaCl, 0.005% TWEEN® 20, 1% DMSO
50 pM SA | 500 nM – 15.3 pM d-Desthiobiotin | 22°C | medium MST power | 80% excitation power

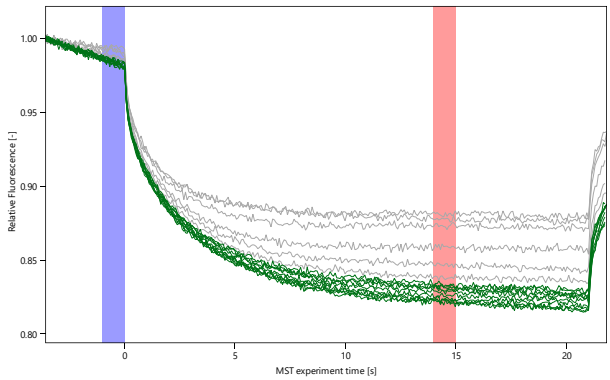
D4. MST Results (Capillary Scan/Time Traces/Dose Response)

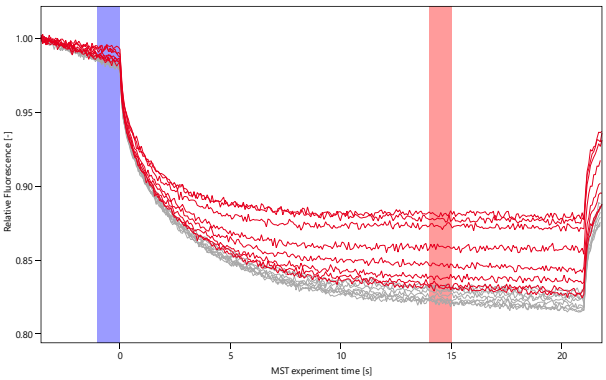
EC_{50}[1] = 0.34 ± 0.07 nM

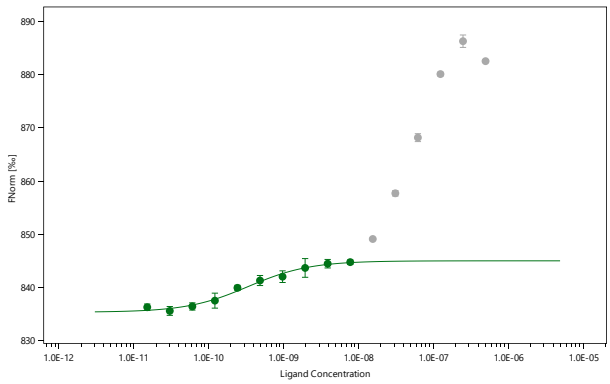
EC_{50}[2] = 48.0 ± 5.5 nM (lower affinity binding sites[3])

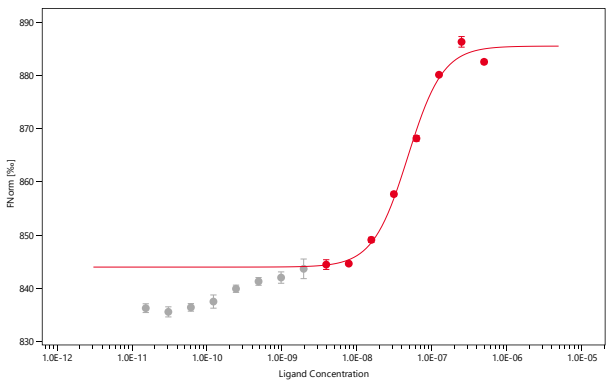












[3] Despite of the preferential labeling of the N-terminal α-amino group, it cannot be completely avoided that some of the SA monomers will still contain labeled lysine residues. In particular, NHS-dye labeling of the ε-amino group of lysine residue K121 has been shown to lead to a steric clash between dye and biotin in the neighboring subunit, decreasing the affinity of that binding site for biotin-4-fluorescein by ~2 orders of magnitude (Jacobson, 2017).

D5. Reference Results/Supporting Results

K_d = 0.66 nM ± 0.18 nM Tryptophan quenching and [3H]-desthiobiotin dissociation
Magalhaes, Protein Science 20, 1145-1154 (2011)

E. Contributors

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