Monolith Protocol MO-P-042

# Streptavidin – Biotin (stoichiometry)

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. MST can be used to determine the stoichiometry of the biotin-streptavidin interaction.

**protein – small molecule interaction | streptavidin | stoichiometry**

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## A1. Target/Fluorescent Molecule

Streptavidin (SA)
uniprot.org/uniprot/P22629

## A2. Molecule Class/Organism

Extracellular proteins
*Streptomyces avidinii*

## A3. Sequence/Formula

```
DPSKESKAQA AVAEAGITGT WYNQLGSTFI VTANPDGSLT GTYESAVGNA ESRYVLTGRY DSTPATDGSG TALGWTVAWK
NNYRNAHSAT TWSGQYVGGA EARINTQWLL TSGTTAANAW KSTLVGHDTF TKVKPSAASI DAAKKAGVNN GNPLDAVQQ
```

## 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 $M^{-1}cm^{-1}$ ($\varepsilon_{280}$)

## 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

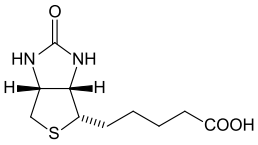
| | | | |
|---|---|---|---|
| Absorbance $A_{280}$ | 0.401 | Protein concentration | 2.10 µM |
| Absorbance $A_{650}$ | 1.677 | Degree-of-labeling (DOL) | 3.19 |

[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

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

## B7. Serial Dilution Preparation

### Dilution series over wide range to narrow down point of saturation

1. Dissolve 12.2 mg biotin in 1 mL of DMSO to obtain a 50 mM solution.
2. Mix 2 µL of 50 mM biotin with 998 µL of DMSO to obtain 1 mL of a 100 µM biotin solution.
3. Mix 2 µL of 100 µM biotin with 198 µL of dilution buffer to obtain 200 µL of a 1 µM biotin solution.
4. Mix 2 µL of DMSO with 198 µL of dilution buffer to obtain 200 µL of a 1% DMSO solution.
5. Prepare a PCR-rack with 16 PCR tubes. Transfer 50 µL of the 1 µM biotin solution into tube **1**. Then, transfer 50 µL of the 1% DMSO solution into tubes **2** to **16**.
6. 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.
7. Mix 25 µL of the ~2 µM SA solution with 975 µL of dilution buffer to obtain 1 mL of a ~50 nM SA solution.
8. Add 50 µL of labeled SA (~50 nM) to each tube from **16** to **1** and mix by pipetting.

### Dilution series over small concentration range to precisely determine stoichiometry

1. Mix 320 µL of 1 µM biotin with 680 µL of dilution buffer to obtain 1 mL of a 320 nM biotin solution.
2. Prepare a PCR-rack with 16 new PCR tubes. Transfer 32 µL, 30 µL, …, 4 µL, 2 µL of the 320 nM biotin solution into tubes **1** to **16**. Then, transfer 2 µL, 4 µL, …, 28 µL, 30 µL of dilution buffer into tubes **2** to **16** to get an equal volume of 32 µL for all samples.
3. Add 32 µL of labeled SA (~50 nM) to each tube from **16** to **1** and mix by pipetting.

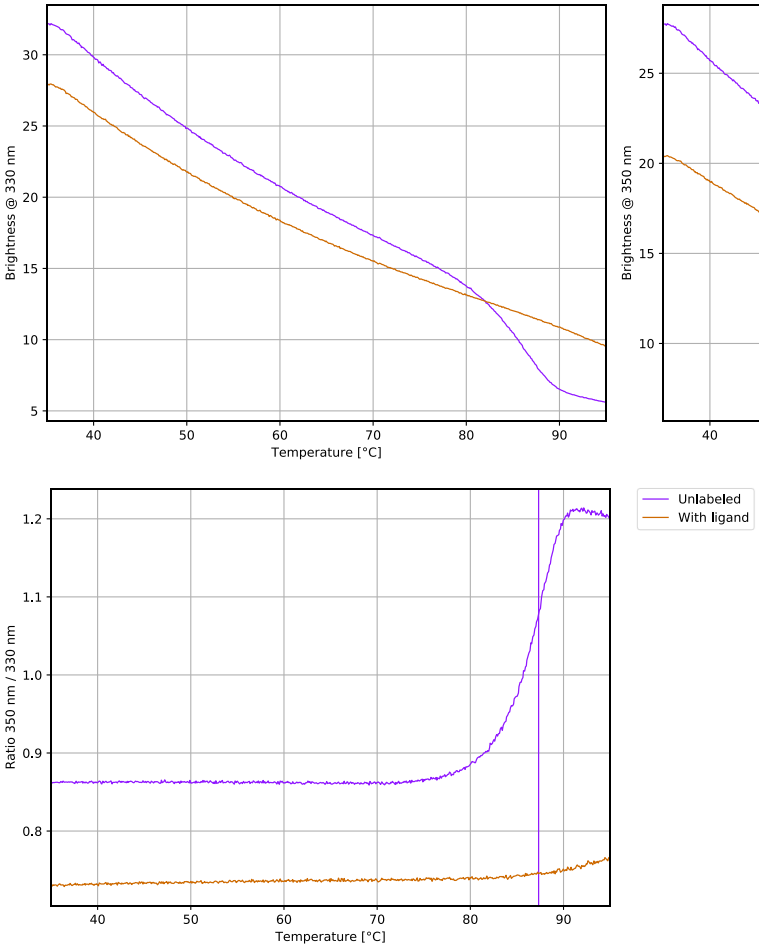
## B8. SD-Test

1. Prepare the SD-mix: Dilute 400 µL of 10% SDS and 40 µL of 1 M DTT in 560 µL water to obtain a solution containing 4% SDS and 40 mM DTT.
2. Transfer 7 µL of the SD-mix to six PCR tubes.
3. Add 7 µL from the remainder of tubes **1** to **3** and **14** to **16** to the tubes containing 7 µL SD-mix. Mix well by pipetting.
4. Place samples on a heat block set to 95°C for 5 minutes to denature the protein, then allow to cool at 25°C for 10 minutes before loading into capillaries.

## C. Applied Quality Checks

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

| | | |
|---|---|---|
| Unlabeled | 5 µL of 2 µM SA + 5 µL of dilution buffer | $T_i$ = 87.3°C |
| With ligand | 5 µL of 2 µM SA + 5 µL of 10 µM biotin | $T_i$ > 95°C[2] |




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

## D1. MST System/Capillaries

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

## D2. MST Software

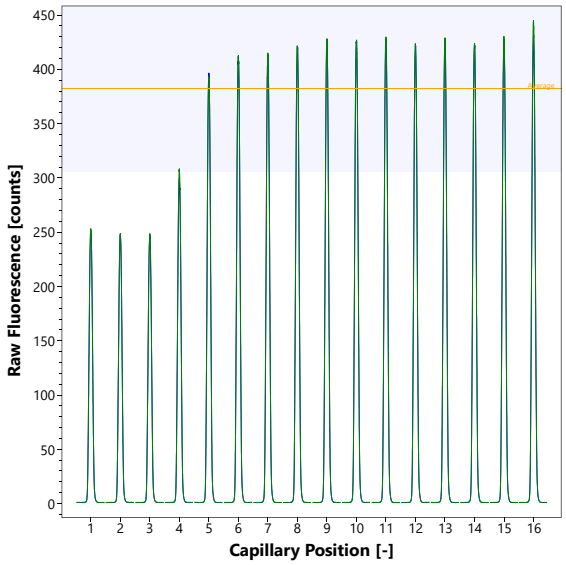
MO.Control v1.6 or higher (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

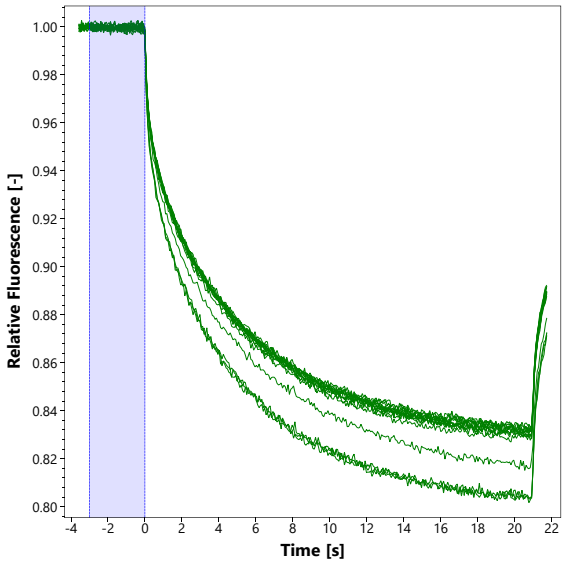
## D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

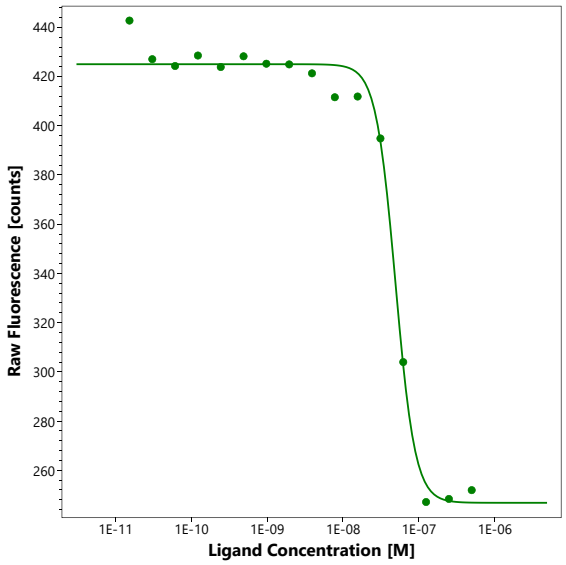
20 mM HEPES, pH 7.4, 150 mM NaCl, 0.005% TWEEN® 20, 1% DMSO
25 nM SA | 500 nM – 15.3 pM and 160 nM – 10 nM biotin | 22°C | medium MST power | 5% excitation power

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

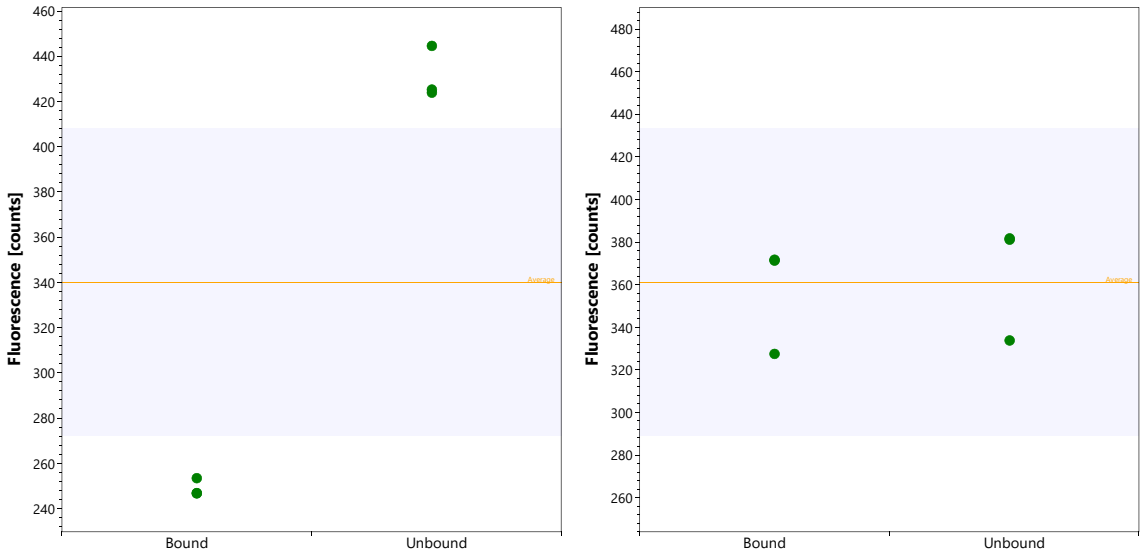
$EC_{50}$ = 49.4 nM



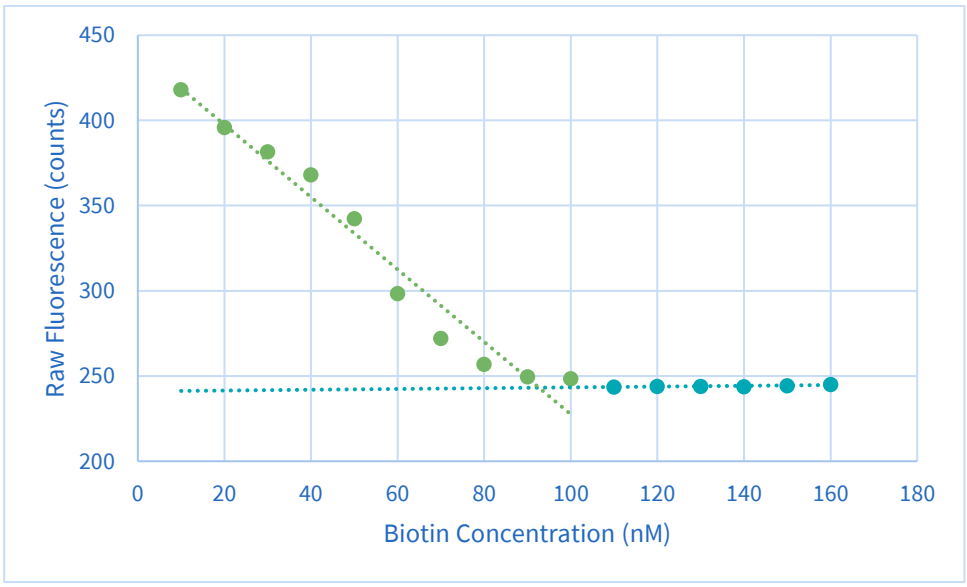



SD-Test:




Intersection of linear fits at ~92 nM biotin (25 nM SA) → 4 : 1 stoichiometry




## D5. Reference Results/Supporting Results

*Streptavidin is a homo-tetramer and each subunit binds biotin with equal affinity.*

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## E. Contributors

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