Monolith Protocol MO-P-019

Bovine Serum Albumin Self-Oligomerization

Bovine serum albumin (also known as BSA) is a serum albumin protein derived from cows. It is often used as a protein concentration standard in lab experiments. At high concentrations, it undergoes a completely reversible self-oligomerization and subsequent structural alteration. Upon dilution, the oligomers dissociate again into a native monomeric state. Polysorbate 20 (TWEEN® 20) can increase the colloidal stability of proteins by interacting with the hydrophobic surface areas of proteins in order to minimize protein-protein interactions and thus protein aggregation.

self-oligomerization | beta-sheet formation | colloidal stability

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A1. Target/Fluorescent Molecule

Bovine serum albumin (BSA)
uniprot.org/uniprot/P02769

A2. Molecule Class/Organism

Serum protein
Bos taurus (Bovine)

A3. Sequence/Formula

```
DTHKSEIAHR FKDLGEEHFK GLVLIAFSQY LQQCPFDEHV KLVNELTEFA KTCVADESHA GCEKSLHTLF GDELCKVASL
RETYGDMADC CEKQEPERNE CFLSHKDDSP DLPKLKPDPN TLCDEFKADE KKFWGKYLYE IARRHPYFYA PELLYYANKY
NGVFQECCQA EDKGACLLPK IETMREKVLA SSARQRLRCA SIQKFGERAL KAWSVARLSQ KFPKAEFVEV TKLVTDLTKV
HKECCHGDLL ECADDRADLA KYICDNQDTI SSKLKECCDK PLLEKSHCIA EVEKDAIPEN LPPLTADFAE DKDVCKNYQE
AKDAFLGSFL YEYSRRHPEY AVSVLLRLAK EYEATLEECC AKDDPHACYS TVFDKLKHLV DEPQNLIKQN CDQFEKLGEY
GFQNALIVRY TRKVPQVSTP TLVEVSRSLG KVGTRCCTKP ESERMPCTED YLSLILNRLC VLHEKTPVSE KVTKCCTESL
VNRRPCFSAL TPDETYVPKA FDEKLFTFHA DICTLPDTEK QIKKQTALVE LLKHKPKATE EQLKTVMENF VAFVDKCCAA
DDKEACFAVE GPKLVVSTQT ALA
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A4. Purification Strategy/Source

Carl Roth GmbH
8076.2

A5. Stock Concentration/Stock Buffer

Powdered

A6. Molecular Weight/Extinction Coefficient

66.5 kDa
43,800 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

Phosphate buffered saline (PBS, pH 7.4)

A8. Labeling Strategy

Monolith Protein Labeling Kit RED – NHS 2nd Generation (MO-L011, NanoTemper Technologies GmbH)
1* Labeling Buffer NHS | 1* Dye RED-NHS 2nd Generation (10 µg) | 1* B-Column

A9. Labeling Procedure

1. Add 3 mL of dilution buffer to 20 mg of BSA to obtain a 100 µM solution. Mix carefully with a pipette to dissolve all protein and avoid creating air bubbles.
2. Prepare 100 µL of a 20 µM BSA solution by mixing 20 µL of the 500 µM solution with 80 µL of Labeling Buffer NHS.
3. Add 25 µL of DMSO to Dye RED-NHS 2nd Generation (10 µg) to obtain a ~600 µM solution. Mix the dye thoroughly by vortexing and make sure that all dye is dissolved.
4. Mix 5 µL of the 600 µM dye solution with 95 µL of Labeling Buffer NHS to obtain 100 µL of a 30 µM dye solution (1.5x protein concentration).
5. Mix BSA and dye in a 1:1 volume ratio (200 µL final volume, 2.5% final DMSO concentration).
6. Incubate for 20 minutes at room temperature in the dark.
7. 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.
8. 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.
9. Add 200 µL of the labeling reaction from step 5 to the center of the column and let sample enter the resin bed completely.
10. Add 400 µL of dilution buffer after the sample has entered and discard the flow through.
11. Place column in a new collection tube, add 500 µL of dilution buffer and collect the eluate.
12. Keep the labeled BSA (~4 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_{205}	7.532	Protein concentration	3.60 µM
Absorbance A_{650}	0.607	Degree-of-labeling (DOL)	0.87

B1. Ligand/Non-Fluorescent Binding Partner

Bovine serum albumin (BSA)
uniprot.org/uniprot/P02769

B2. Molecule Class/Organism

Serum albumin
Bos taurus (Bovine)

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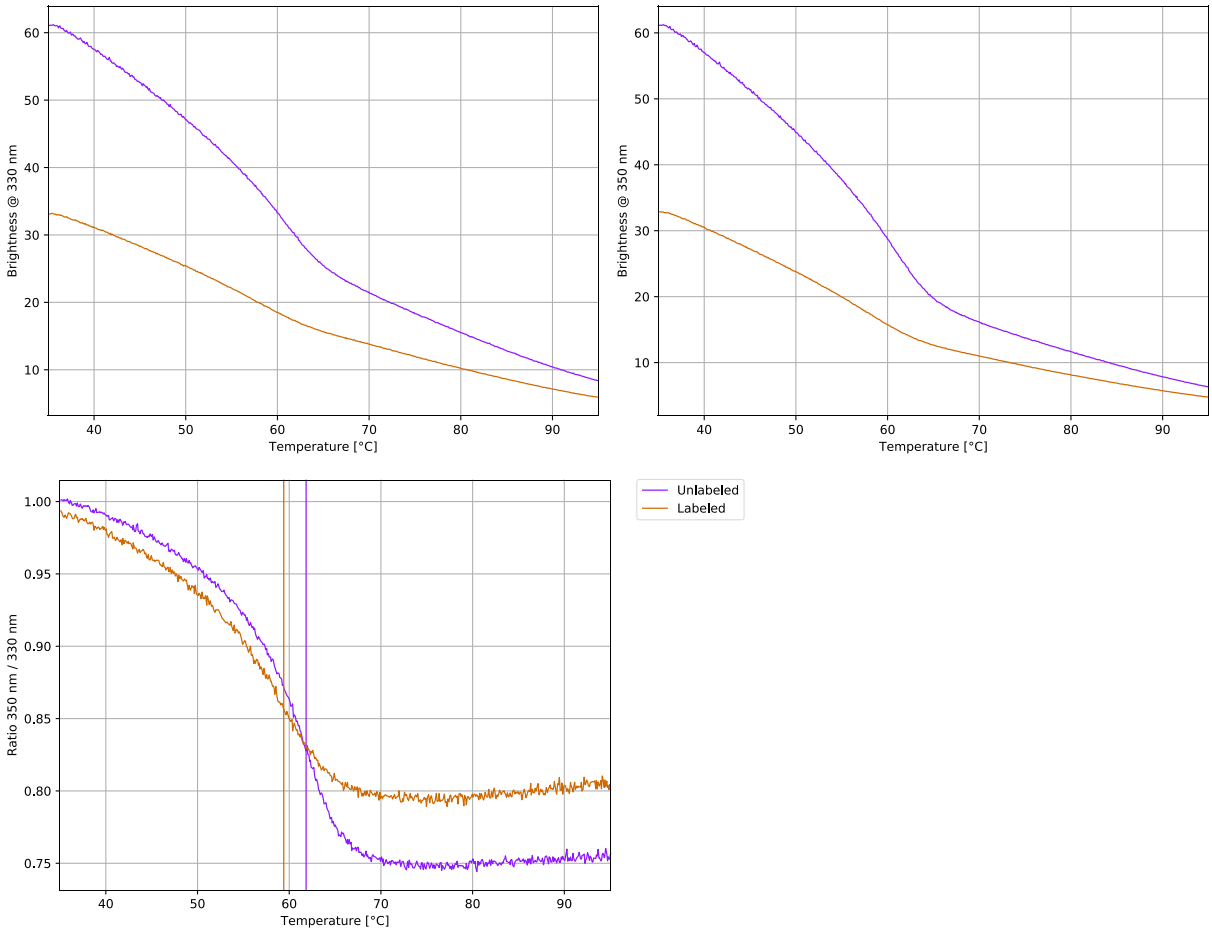
B7. Serial Dilution Preparation

1. Resuspend 20 g of TWEEN® 20 in 180 mL of ddH_2O to obtain a 10% TWEEN® 20 stock solution.
2. Mix 8 µL of the 10% TWEEN® 20 stock solution with 492 µL of dilution buffer in a 0.5 mL tube to obtain 500 µL of a 0.16% TWEEN® 20 solution.
3. Prepare seven more 0.5 mL tubes and fill each of them with 250 µL of dilution buffer. Then, prepare a 1:1 serial dilution of the 0.16% TWEEN® 20 solution by transferring 250 µL from tube to tube to obtain concentrations between 0.08% and 0.00125% TWEEN® 20. Mix carefully by pipetting up and down.
4. Prepare a PCR-rack with 16 PCR tubes. Transfer 20 µL of the 100 µM BSA solution into tube **1**. Then, transfer 10 µL of dilution buffer into tubes **2** to **16**.
5. Prepare a 1:1 serial dilution by transferring 10 µL from tube to tube. Mix carefully by pipetting up and down. Remember to discard 10 µL from tube **16** to get an equal volume of 10 µL for all samples.
6. Mix 1 µL of labeled BSA (~4 µM) with 199 µL of the 0.01% TWEEN® 20 solution to obtain 200 µL of ~20 nM BSA.
7. Add 10 µL of labeled BSA (~20 nM) to each tube from **16** to **1** and mix by pipetting.
8. Incubate for 5 minutes at room temperature in the dark before loading capillaries.
9. Repeat steps 4 to 8 for all remaining TWEEN® 20 concentrations.

C. Applied Quality Checks

Validation of structural integrity of labeled BSA using Tycho NT.6:
nanotempertech.com/tycho

Unlabeled	4 µL of 100 µM BSA + 96 µL of dilution buffer	T_i = 61.8°C
Labeled	10 µL of B-column eluate (~4 µM BSA) in dilution buffer	T_i = 59.4°C



D1. MST System/Capillaries

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

D2. MST Software

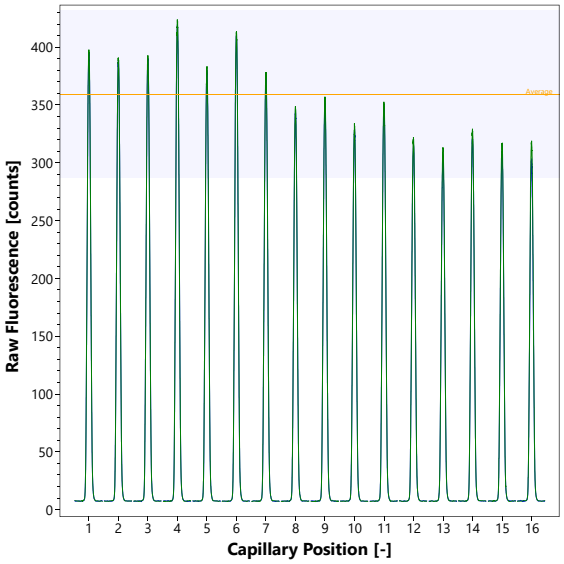
MO.Control v1.6 (NanoTemper Technologies GmbH)
nanotempertech.com/monolith-mo-control-software

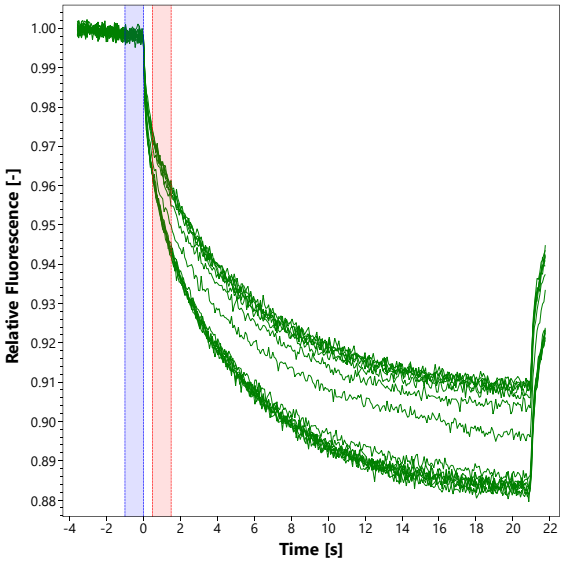
D3. MST Experiment (Assay Buffer/Concentrations/Temperature/MST Power/Excitation Power)

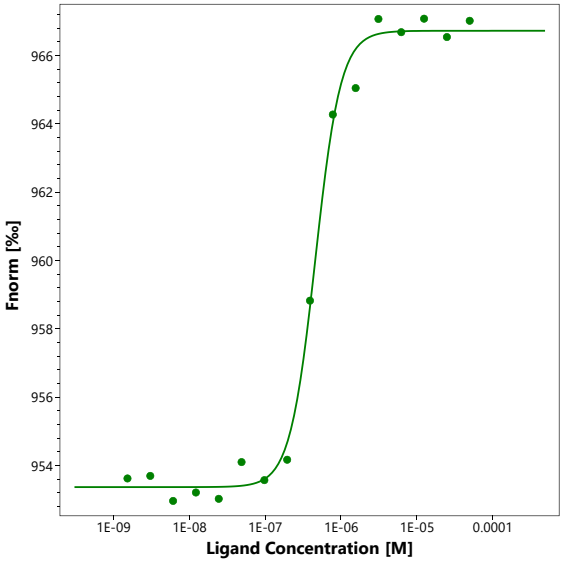
Phosphate buffered saline (PBS, pH 7.4), 0.08% – 0.000625% TWEEN® 20
10 nM (labeled) BSA | 1.5 nM – 50 µM (unlabeled) BSA | 22°C | medium MST power | 50% excitation power

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

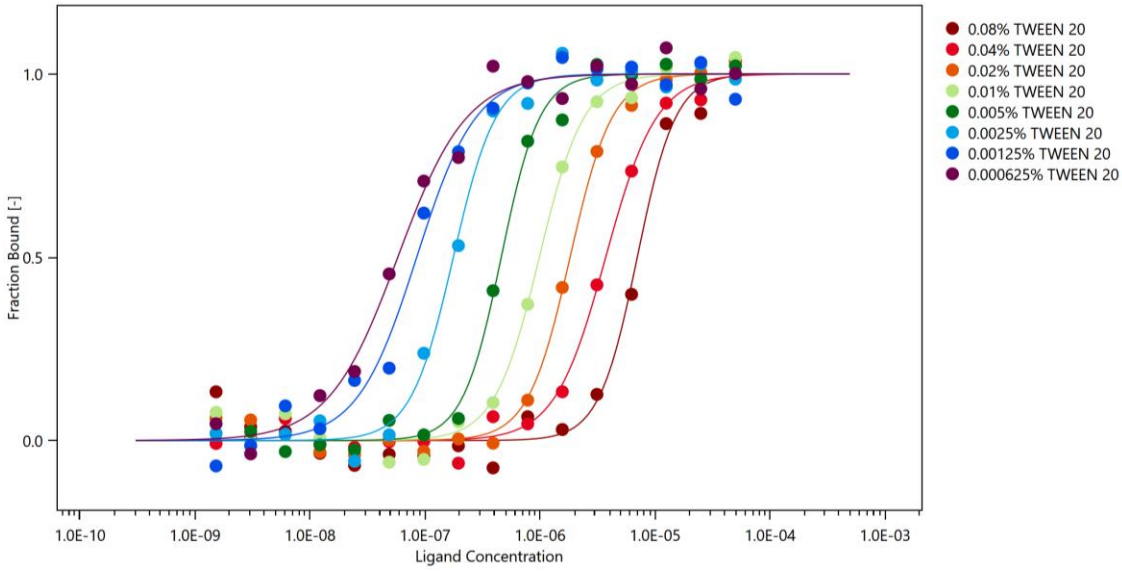
EC_{50} = 460 nM (0.005% TWEEN® 20)







TWEEN® 20 (%)	0.08	0.04	0.02	0.01	0.005	0.0025	0.00125	0.000625
EC_{50} (µM)	7.01	3.73	1.83	0.987	0.460	0.176	0.085	0.058



TWEEN® 20 delays the self-oligomerization of BSA to higher BSA concentrations.

D5. Reference Results/Supporting Results

Various ground state oligomers of serum albumins in the concentration range 10–150 µM that reversible dissociate into a native monomeric state upon dilution (spectroscopic techniques and scanning electron microscopy, SEM).
Bhattacharya et al., Langmuir 30 (2014) 14894–14904

E. Contributors

Andreas Langer[1]

[1] NanoTemper Technologies GmbH, München, Germany | nanotempertech.com