Prometheus Protocol PR-P-016

Optimization of TCEP Reduction Conditions

Bovine serum albumin (also known as BSA) is a serum albumin protein derived from cows, which is often used as a protein concentration standard in lab experiments. Among its 583 amino acid residues, it contains 17 intrachain disulfide bonds as well as one free thiol group, which can be used for site-specific labeling with a thiol-reactive (maleimide) fluorescent dye. Tris(2-carboxyethyl)phosphine (TCEP) is a widely used reducing agent for the reduction of disulfide bonds on proteins. High TCEP concentrations and/or long incubation times can impair the structural integrity of BSA by reducing structurally important intrachain disulfide bonds.

structural integrity | reducing agents | TCEP

A1. Target/Fluorescent Molecule

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

A2. Molecular Class/Organism

Serum protein
Bos taurus (Bovine)

A3. Sequence/Formula

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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

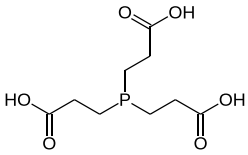
66.5 kDa
43,800 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

Phosphate buffered saline (PBS, pH 7.4)

B1. Ligand/Non-Fluorescent Binding Partner

Tris(2-carboxyethyl)phosphine (TCEP)



B2. Molecule Class/Organism

Reducing agent

B3. Sequence/Formula

$C_9H_{15}O_6P$

B4. Purification Strategy/Source

Sigma-Aldrich GmbH
C4706

B5. Stock Concentration/Stock Buffer

0.5 M
pH 7.0 (aqueous solution; pH adjusted with ammonium hydroxide)

B6. Molecular Weight/Extinction Coefficient

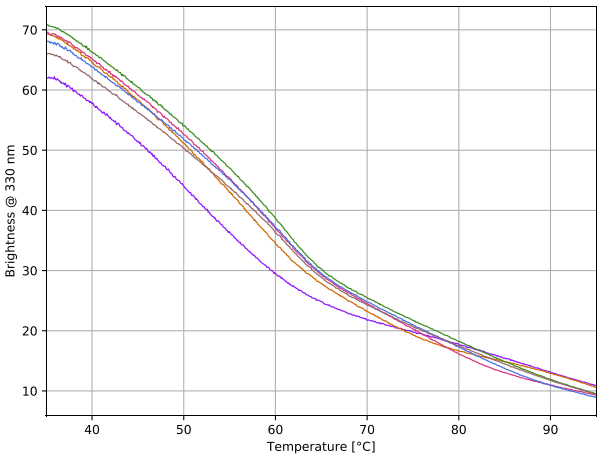
286.65 Da

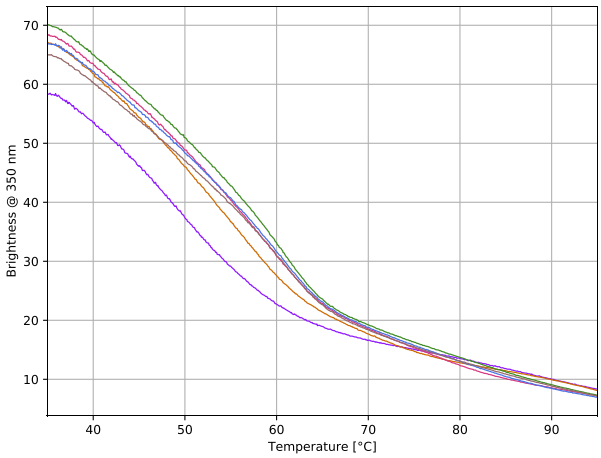
C. Applied Quality Checks

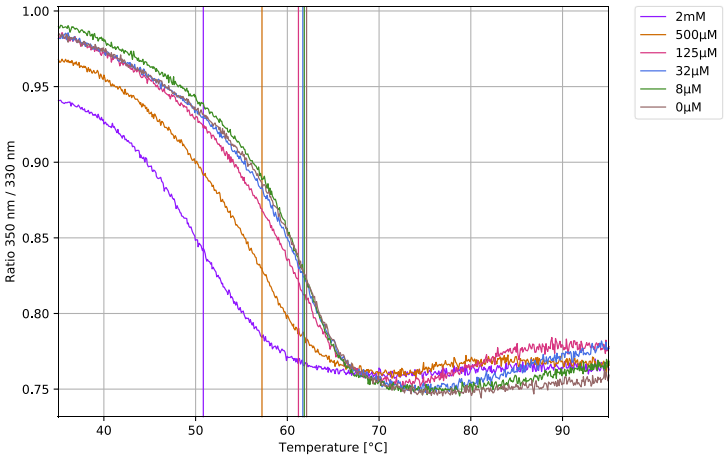
Validation of structural integrity of BSA and TCEP activity using Tycho NT.6 (**directly** after mixing, see D3):

nanotempertech.com/tycho

2mM	5 µM BSA, 2 mM TCEP, tube **1**	T_i = 50.8°C
500µM	5 µM BSA, 500 µM TCEP, tube **3**	T_i = 57.2°C
125µM	5 µM BSA, 125 µM TCEP, tube **5**	T_i = 61.2°C
32µM	5 µM BSA, 32 µM TCEP, tube **7**	T_i = 61.7°C
8µM	5 µM BSA, 8 µM TCEP, tube **9**	T_i = 61.8°C
0µM	5 µM BSA, tube **11**	T_i = 62.1°C

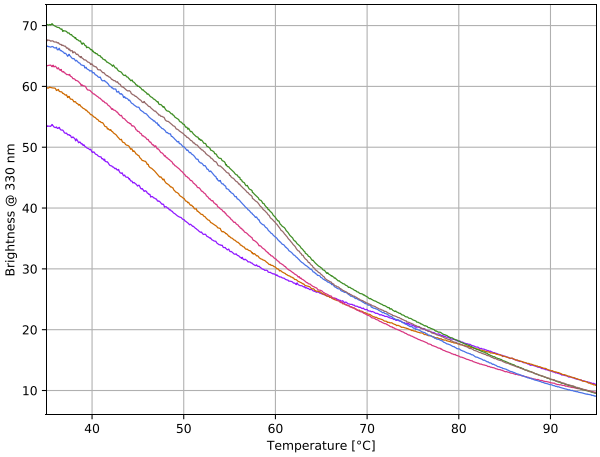


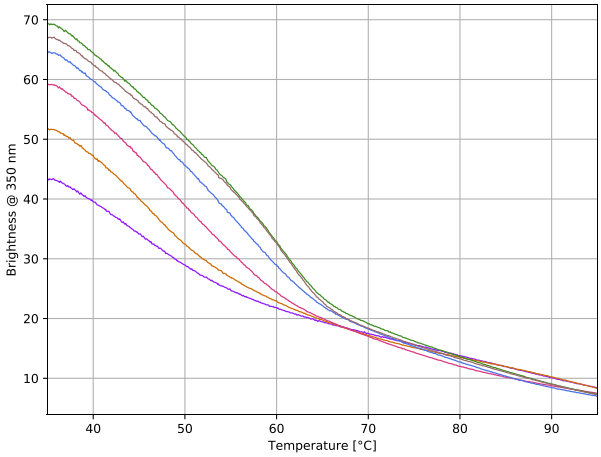


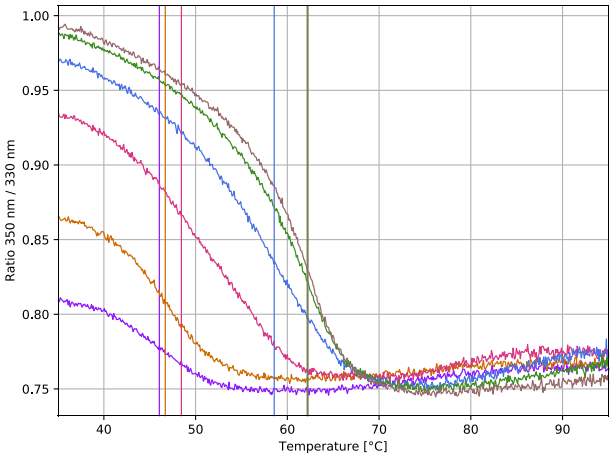


Validation of structural integrity of BSA and TCEP activity using Tycho NT.6 (after **2 hours** of incubation):
nanotempertech.com/tycho

2mM	5 µM BSA, 2 mM TCEP, tube **1**	T_i = 46.0°C
500µM	5 µM BSA, 500 µM TCEP, tube **3**	T_i = 46.7°C
125µM	5 µM BSA, 125 µM TCEP, tube **5**	T_i = 48.5°C
32µM	5 µM BSA, 32 µM TCEP, tube **7**	T_i = 58.6°C
8µM	5 µM BSA, 8 µM TCEP, tube **9**	T_i = 62.2°C
0µM	5 µM BSA, tube **11**	T_i = 62.3°C

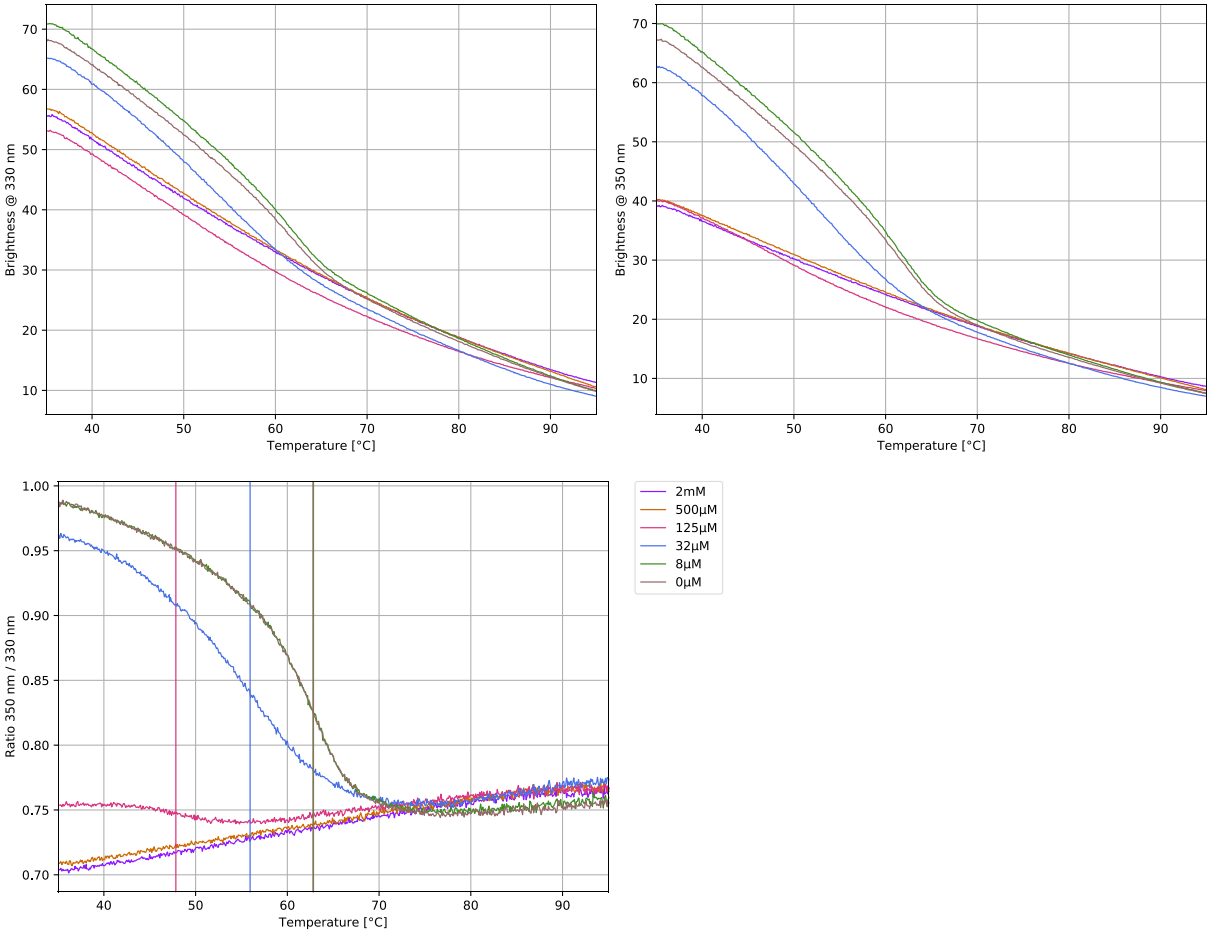






Validation of structural integrity of BSA and TCEP activity using Tycho NT.6 (after **24 hours** of incubation):
nanotempertech.com/tycho

2mM	5 µM BSA, 2 mM TCEP, tube **1**	T_i = N/A
500µM	5 µM BSA, 500 µM TCEP, tube **3**	T_i = N/A
125µM	5 µM BSA, 125 µM TCEP, tube **5**	T_i = 47.8°C
32µM	5 µM BSA, 32 µM TCEP, tube **7**	T_i = 55.9°C
8µM	5 µM BSA, 8 µM TCEP, tube **9**	T_i = 62.8°C
0µM	5 µM BSA, tube **11**	T_i = 62.8°C



D1. nanoDSF System/Capillaries

Prometheus NT.48 (NanoTemper Technologies GmbH)
High Sensitivity Capillaries Prometheus NT.48 nanoDSF Grade (PR-C006, NanoTemper Technologies GmbH)

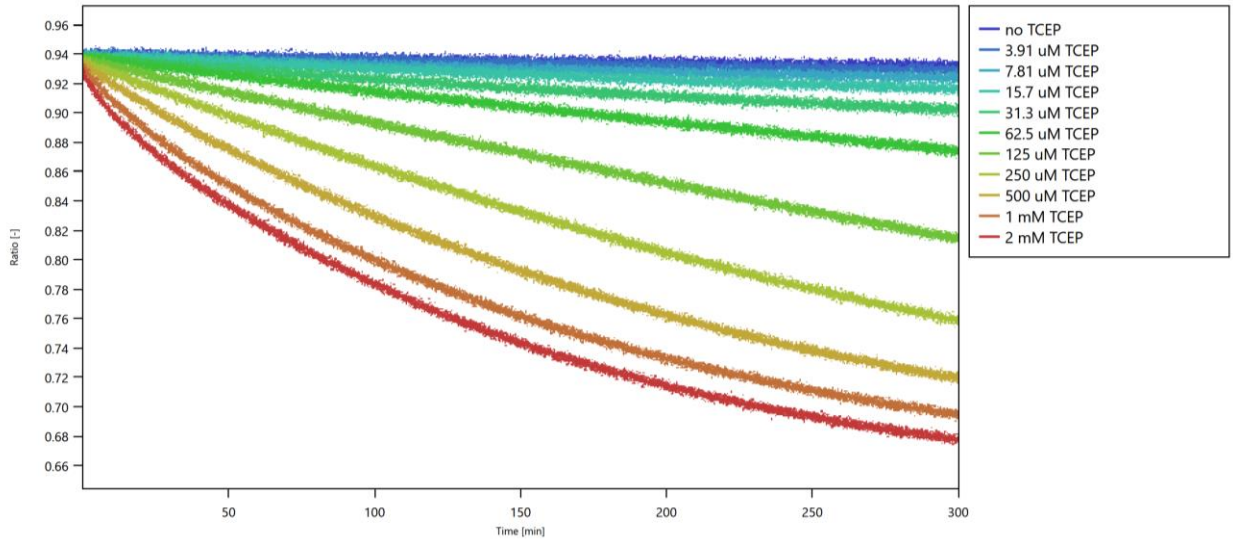
D2. nanoDSF Software

PR.TimeControl v1.0.2 (NanoTemper Technologies GmbH)
nanotempertech.com/prometheus-software

D3. nanoDSF Experiment

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. Mix 100 µL of 100 µM BSA with 900 µL of dilution buffer to obtain 1 mL of a 10 µM BSA solution.
3. Prepare a PCR-rack with 11 PCR tubes. Mix 1.3 µL of 0.5 M TCEP with 158.6 µL of dilution buffer in tube **1**. Then, transfer 80 µL of dilution buffer into tubes **2** to **11**.
4. Prepare a 1:1 serial dilution by transferring 80 µL from tube to tube (**omit** tube **11**, which will be the buffer control). Mix carefully by pipetting up and down. Remember to discard 80 µL from tube **10** to get an equal volume of 80 µL for all samples.
5. Add 80 µL of BSA (10 µM) to each tube from **11** to **1** and mix by pipetting.
6. Start a new session of the *PR.TimeControl* software.
7. Go to 'Measurement Scan' and prepare a run with the following settings:
    a. Only capillaries 1 – 11 selected
    b. Isothermal
    c. 25°C
    d. 5 hours
    e. 50% excitation power
8. Load capillaries from each of the 11 tubes and place them on positions 1 – 11 of the Prometheus capillary tray.
9. Place the magnetic lid to fix the capillary.
10. Start the measurement.

D4. nanoDSF Results



Based on these results, TCEP concentrations >50 µM and incubation times >30 minutes should be avoided.

D5. Reference Results/Supporting Results

Fully disulfide-reduced BSA was found to lose its native structure and ligand-binding ability.
Borzova et al., Int J of Bio Macromolecules 80 (2015) 130–138

E. Contributors

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