Prometheus Protocol PR-P-009

Coaggregation and Cross-Seeding

As a lot of different proteins coexist in cells, it is important to understand how an aggregating protein can affect the aggregation of another protein in its vicinity. Amyloid formation is typically monitored in Thioflavin T (ThT) assays, although the exact mechanism of action and whether the dye itself affects the kinetics of aggregation is still debated. In this protocol, the *in vitro* coaggregation and cross-seeding of lysozyme and bovine serum albumin (also known as BSA) during their amyloid formation is followed in real time without the need of a dye.

coaggregation | cross-seeding | amyloid formation

A1. Target/Fluorescent Molecule

Lysozyme
uniprot.org/uniprot/B8YK79

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

A2. Molecule Class/Organism

Glycoside hydrolases
Gallus gallus (Chicken)

Serum protein
Bos taurus (Bovine)

A3. Sequence/Formula

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KVFGRCELAA AMKRHGLDNY
RGYSLGNWVC AAKFESNFNT
QATNRNTDGS TDYGILQINS
RWWCNDGRTP GSRNLCNIPC
SALLSSDITA SVNCAKKIVS
DGNGMNAWVA WRNRCKGTDV
QAWIRGCRL
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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

Sigma-Aldrich GmbH
L6876

Carl Roth GmbH
8076.2

A5. Stock Concentration/Stock Buffer

100 µM | 1.43 mg/mL
20 mM HEPES, pH 7.4, 150 mM NaCl

100 µM | 6.65 mg/mL
20 mM HEPES, pH 7.4, 150 mM NaCl

A6. Molecular Weight/Extinction Coefficient

14.3 kDa	66.5 kDa
37,970 $M^{-1}cm^{-1}$ (ε_{280})	43,800 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

20 mM HEPES, pH 7.4, 150 mM NaCl

D1. nanoDSF System/Capillaries

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

D2. nanoDSF Software

PR.ThermControl v2.1 | PR.TimeControl v1.0.2 (NanoTemper Technologies GmbH)
nanotempertech.com/prometheus-software

D3. nanoDSF Experiment

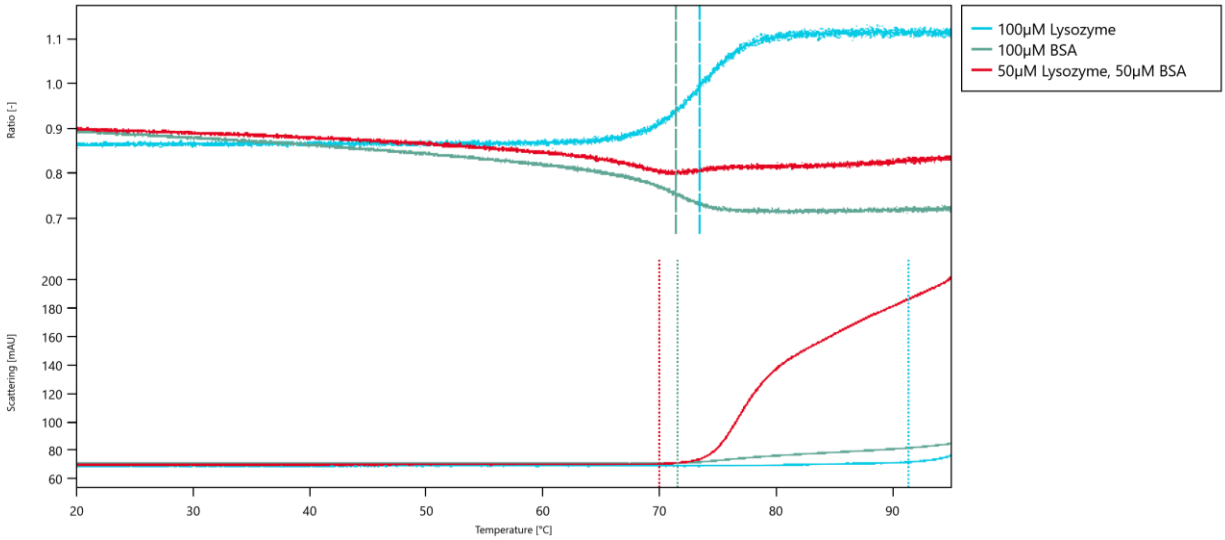
1. Prepare 3 PCR tubes. Fill 25 µL of 100 µM lysozyme into tube **1**, 25 µL of 100 µM BSA into tube **2**, then mix 12.5 µL of 100 µM lysozyme and 12.5 µL of 100 µM BSA in tube **3** to obtain a 50 µL of a 50 µM lysozyme, 50 µM BSA mix.
2. Start a new session of the *PR.ThermControl* software.
3. Completely fill three capillaries from the three tubes, place them on positions 1 – 3 of the capillary tray and place the magnetic lid to fix the capillaries.
4. Go to 'Melting Scan' and prepare a run with the following settings:
    a. Capillaries 1 – 3 selected
    b. 1.0°C/min
    c. 20°C – 95°C
    d. 2% excitation power
5. Start the measurement.
6. After the measurement is finished, start a new session of the *PR.TimeControl* software.
7. Completely fill three capillaries from the three tubes, place them on positions 1 – 3 of the capillary tray and place the magnetic lid to fix the capillaries.
8. Go to 'Melting Scan' and prepare a run with the following settings:
    a. Capillaries 1 – 3 selected
    b. Isothermal
    c. 70°C[1]
    d. 1200 min (20 hours, overnight)
    e. 2% excitation power
9. Start the measurement.

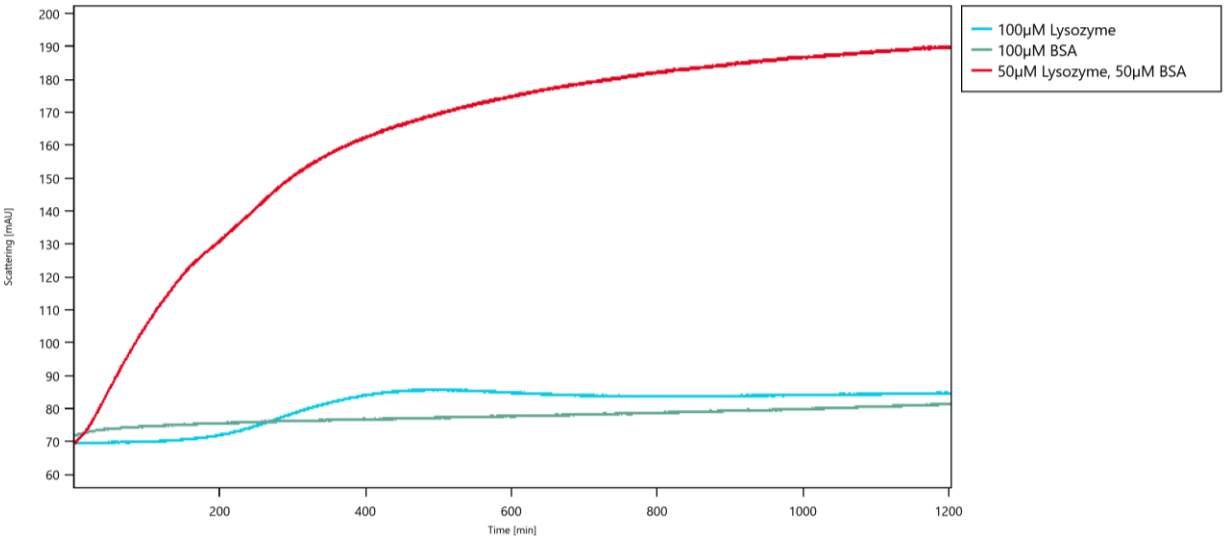
[1] The temperature is chosen so that it is close to the T_m values of lysozyme and BSA.

D4. nanoDSF Results

T_m = 73.4°C (lysozyme) | T_m = 71.5°C (BSA)

T_{agg} = 91.4°C (lysozyme) | T_{agg} = 71.6°C (BSA) | T_{agg} = 70.0°C (lysozyme & BSA mix)





D5. Reference Results/Supporting Results

Thioflavin T assay

Dubey et al., Biochemistry 2014, 53 (51), 8001–8004

E. Contributors

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