Prometheus Protocol PR-P-017

Suppression of Protein Aggregation by L-Arginine

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. L-Arginine is one of the most commonly used and most generally applicable suppressors of protein aggregation.

coaggregation | cross-seeding | amyloid formation | aggregation suppression

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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

```
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

20 mg
Lyophilized powder

128 µg
Lyophilized powder

A6. Molecular Weight/Extinction Coefficient

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

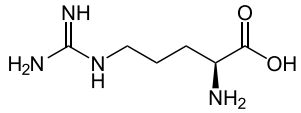
66.5 kDa
43,800 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

Phosphate buffered saline (PBS, pH 7.4)

B1. Ligand/Non-Fluorescent Binding Partner

(S)-2-Amino-5-guanidinopentanoic acid (L-Arginine)



B2. Molecule Class/Organism

α-amino acid

B3. Sequence/Formula

$C_6H_{14}N_4O_2$

B4. Purification Strategy/Source

Sigma Aldrich GmbH
A5006

B5. Stock Concentration/Stock Buffer

100 g
Powdered

B6. Molecular Weight/Extinction Coefficient

174.20 Da

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

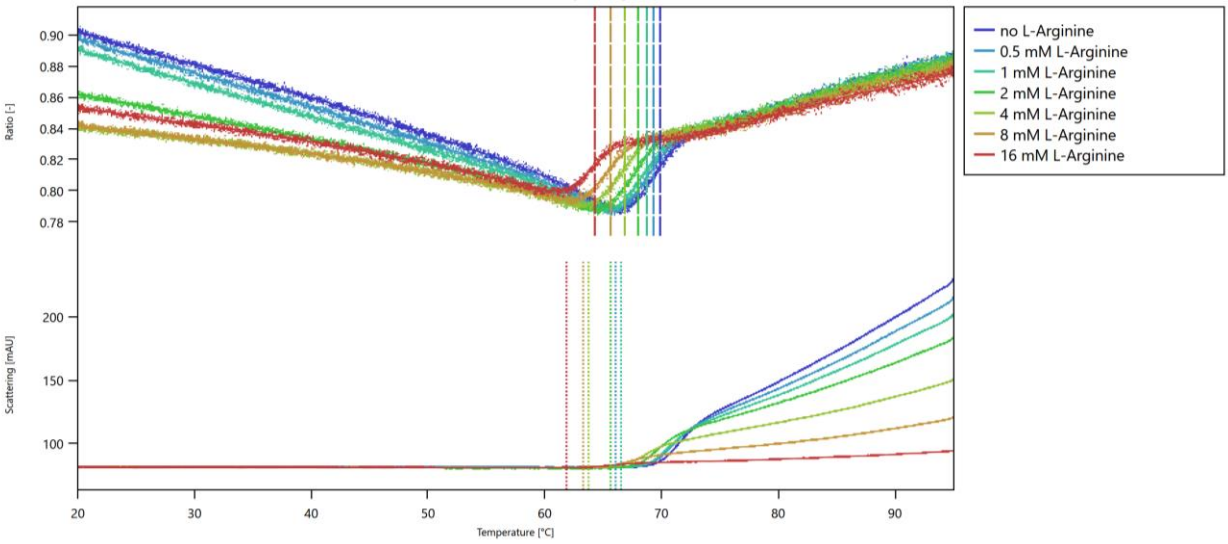
D3. nanoDSF Experiment

1. Dissolve 87 mg of L-Arginine in 1 mL of dilution buffer to obtain a 500 mM L-Arginine solution.
2. 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.
3. Dissolve 128 µg of lysozyme in 89.6 µL of the 100 µM BSA solution to obtain a 100 µM lysozyme, 100 µM BSA solution.
4. Prepare a PCR-rack with 7 PCR tubes. Mix 1.3 µL of the 500 mM L-Arginine solution with 18.7 µL of dilution buffer in tube **1**. Then, transfer 10 µL of dilution buffer into tubes **2** to **7**.
5. Prepare a 1:1 serial dilution by transferring 10 µL from tube to tube (**omit** tube **7**, which will be the buffer control). Mix carefully by pipetting up and down. Remember to discard 10 µL from tube **6** to get an equal volume of 20 µL for all samples.
6. Add 10 µL of the 100 µM lysozyme, 100 µM BSA solution to each tube from **8** to **1** and mix by pipetting.
7. Start a new session of the *PR.TimeControl* software.
8. Go to 'Measurement Scan' and prepare a run with the following settings:
    a. Capillaries 1 – 7 selected
    b. 1.0°C/min
    c. 20°C – 95°C
    d. 10% excitation power
9. Load capillaries from each of the 7 tubes and place them on positions 1 – 7 of the Prometheus capillary tray.
10. Place the magnetic lid to fix the capillary.
11. Start the measurement.
12. After the measurement is finished, start a new session of the *PR.TimeControl* software.
13. Set the instrument temperature to 60°C on the touch display.
14. Go to 'Measurement Scan' and prepare a run with the following settings:
    a. Capillaries 1 – 7 selected
    b. Isothermal
    c. 70°C
    d. 2 hours
    e. 10% excitation power
15. Load capillaries from each of the 7 tubes and place them on positions 1 – 7 of the Prometheus capillary tray.
16. Place the magnetic lid to fix the capillary.
17. Start the measurement.

D4. nanoDSF Results

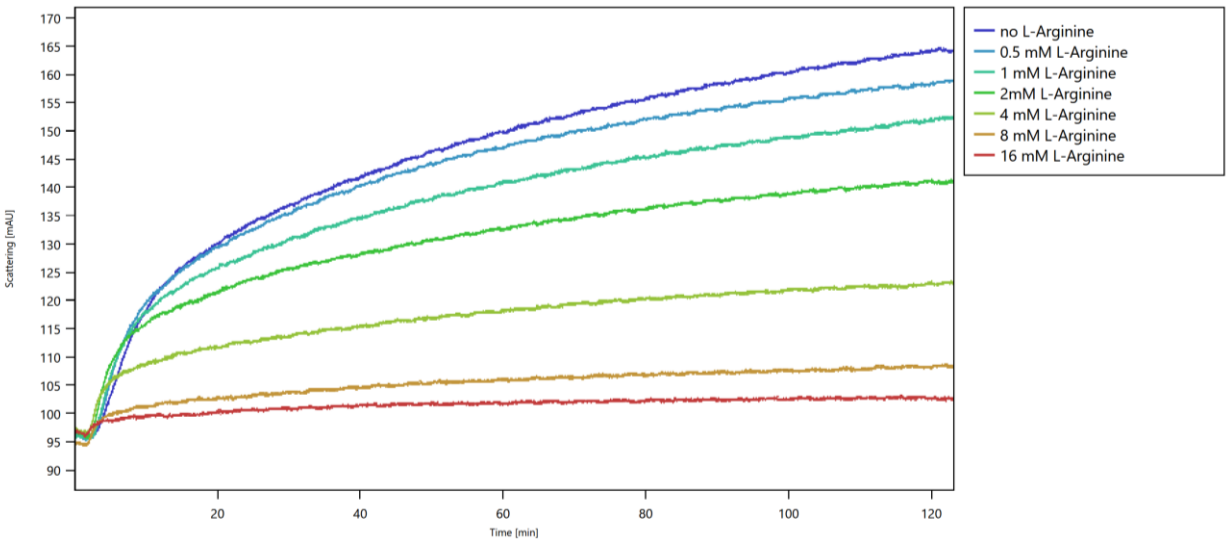
L-Arginine lowers the T_m but suppresses aggregation.[1]

PR.ThermControl



L-Arginine	0 mM	0.5 mM	1 mM	2 mM	4 mM	8 mM	16 mM
T_m	69.8°C	69.3°C	68.8°C	68.0°C	66.9°C	65.7°C	64.3°C

PR.TimeControl



D5. Reference Results/Supporting Results

Thioflavin T assay

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

[1] See Das et al., PLoS ONE 11 (2007) for more information on the mechanism of aggregation suppression by arginine.

E. Contributors

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