Prometheus Protocol PR-P-002

Chemical Unfolding of Lysozyme

Lysozyme is an enzyme that prevents bacterial infections by attacking peptidoglycan, a component of certain bacterial cell walls. Peptidoglycan is composed of the repeating amino sugars N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM), which are crosslinked by peptide bridges. Lysozyme hydrolyzes the bond between NAG and NAM, increasing the bacteria's permeability and causing the bacteria to burst. It is widely distributed in plants and animals. The majority of the lysozyme used in research is purified from hen egg whites.

chemical unfolding | guanidine hydrochloride | ΔG | c_{50}

A1. Target/Fluorescent Molecule

Lysozyme
uniprot.org/uniprot/B8YK79

A2. Molecule Class/Organism

Glycoside hydrolases
Gallus gallus (Chicken)

A3. Sequence/Formula

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

A4. Purification Strategy/Source/Batch-No.

Sigma-Aldrich GmbH
L6876

A5. Stock Concentration/Stock Buffer

32 µg lyophilized powder

A6. Molecular Weight/Extinction Coefficient

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

A7. Dilution Buffer

Phosphate-buffered saline (PBS), pH 7.4

D1. nanoDSF System/Capillaries

Prometheus NT.48 (NanoTemper Technologies GmbH)
Standard Capillaries Prometheus NT.48 nanoDSF Grade (PR-C002, NanoTemper Technologies GmbH)

D2. nanoDSF Software

PR.ChemControl v1.4 (NanoTemper Technologies GmbH)
nanotempertech.com/prometheus-software

D3. nanoDSF Experiment

1. Dissolve 764 mg of guanidine hydrochloride (GuHCl, MW 95.53) in 1 mL of dilution buffer to obtain an 8 M solution. Heat the solution until all GuHCl is dissolved.
2. Prepare 24 small PCR tubes according to the following table:

Tube	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
8 M GuHCl (µL):	24	23	22	21	20	19	18	17	16	15	14	13
Dilution buffer (µL):	6	7	8	9	10	11	12	13	14	15	16	17
Final GuHCl (M):	6	5.75	5.5	5.25	5	4.75	4.5	4.25	4	3.75	3.5	3.25

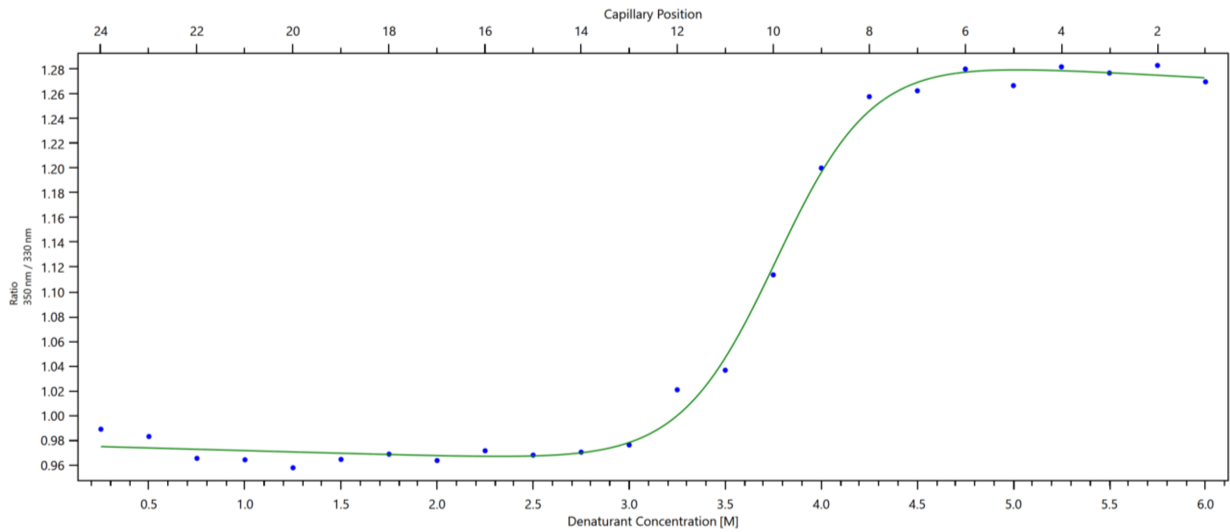
Tube	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
8 M GuHCl (µL):	12	11	10	9	8	7	6	5	4	3	2	1
Dilution buffer (µL):	18	19	20	21	22	23	24	25	26	27	28	29
Final GuHCl (M):	3	2.75	2.5	2.25	2	1.75	1.5	1.25	1	0.75	0.5	0.25

3. Resuspend 32 µg lysozyme in 64 µL of dilution buffer to obtain a 0.5 mg/mL solution.
4. Add 2 µL of this solution to each vial from step 2, mix well and incubate for at least 1 hour[1] at room temperature to ensure equilibrium.
5. Load 24 capillaries with the solutions from step 3 and place them on positions 1 to 24 of the capillary tray according to the vial number. Place the magnetic lid to fix the capillaries.
6. Start a new session of the *PR.ChemControl* software.
7. Go to 'Plan' and prepare a run with the following settings:
    a. Start concentration: 6 M
    b. End concentration: 0.25 M
8. Start the measurement.

[1] As Lysozyme is a quite small protein, equilibration takes place rather fast and an incubation time of about 1 hour is sufficient. Larger biomolecules such as monoclonal antibodies often require a much longer incubation time to equilibrate sufficiently and at least overnight incubation (better 24 – 72 hours) is recommended. Performing the measurement too early will lead to incorrect ΔG and c_{50} values.

D4. nanoDSF Results

ΔG = 36.5 kJ/mol | c_{50} = 3.8 M



D5. Reference Results/Supporting Results

ΔG = 37.2 kJ/mol | c_{50} = 4.2 M   Absorbance measurements

Ahmand et al., Biochem J 287 (1992) 481-485

E. Contributors

Andreas Langer[2]

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