Prometheus Protocol PR-P-019

Chemical Unfolding of a Monoclonal Antibody

Herceptin (Trastuzumab) is a monoclonal antibody used to treat breast cancer. In this protocol, the chemical stability is determined using a chemical unfolding measurement with PR.ChemControl.

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

A1. Target/Fluorescent Molecule

Herceptin (Trastuzumab)

A2. Molecule Class/Organism

Monoclonal antibody

A3. Sequence/Formula

Heavy chain

```
EVQLVESGGG LVQPGGSLRL SCAASGFNIK DTYIHWVRQA PGKGLEWVAR IYPTNGYTRY ADSVKGRFTI SADTSKNTAY
LQMNSLRAED TAVYYCSRWG GDGFYAMDYW GQGTLVTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS
WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG
PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK
EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSREE MTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV
LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPG
```

Light chain

```
DIQMTQSPSS LSASVGDRVT ITCRASQDVN TAVAWYQQKP GKAPKLLIYS ASFLYSGVPS RFSGSRSGTD FTLTISSLQP
EDFATYYCQQ HYTTPPTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ
ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
```

A4. Purification Strategy/Source/Batch-No.

N/A

A5. Stock Concentration/Stock Buffer

120 mg/mL | 825 µM

A6. Molecular Weight/Extinction Coefficient

145.5 kDa
225,000 $M^{-1}cm^{-1}$ (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. Add 145 µL of 1x PBS to 20 µL of 825 µM Herceptin to obtain 165 µL of a 100 µM Herceptin solution.
2. Prepare 1 mL of a 8 M solution of Guanidine Hydrochloride (GuHCl) in ddH_2O.
3. Prepare two 1.5 mL tubes and label them with **A** and **B**.
4. Solution **A** – 1280 µL of a 5 µM Herceptin solution in **5 M GuHCl**, 1x PBS:
   Mix 800 µL of 8 M GuHCl with 200 µL of a 5x PBS stock solution. Then, add 216 µL of 1x PBS and 64 µL of the 100 µM Herceptin solution. Mix carefully using a pipette.
5. Solution **B** – 1280 µL of a 5 µM Herceptin solution in **0.2 M GuHCl**, 1x PBS:
   Mix 32 µL of 8 M GuHCl with 768 µL of ddH_2O and 200 µL of a 5x PBS stock solution. Then, add 216 µL of 1x PBS and 64 µL of the 100 µM Herceptin solution. Mix carefully using a pipette.
6. Prepare 48 small PCR tubes according to the following table:

Tube #	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Solution **A** (µL):	48	47	46	45	44	43	42	41	40	39	38	37
Solution **B** (µL):	0	1	2	3	4	5	6	7	8	9	10	11
Final GuHCl (M):	5	4.9	4.8	4.7	4.6	4.5	4.4	4.3	4.2	4.1	4.0	3.9

Tube #	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Solution **A** (µL):	36	35	34	33	32	31	30	29	28	27	26	25
Solution **B** (µL):	12	13	14	15	16	17	18	19	20	21	22	23
Final GuHCl (M):	3.8	3.7	3.6	3.5	3.4	3.3	3.2	3.1	3.0	2.9	2.8	2.7

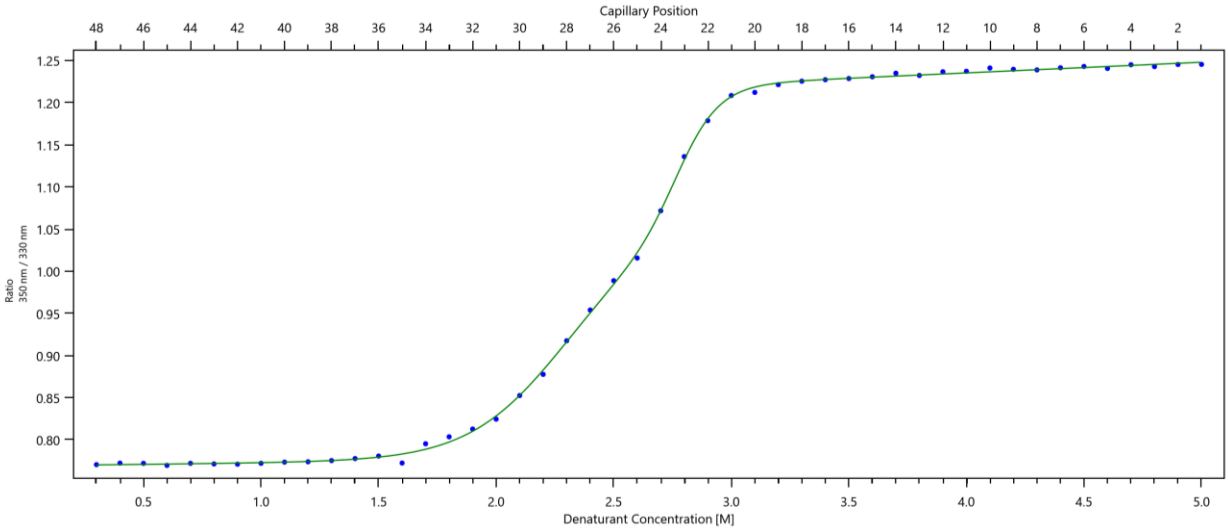
Tube #	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**
Solution **A** (µL):	24	23	22	21	20	19	18	17	16	15	14	13
Solution **B** (µL):	24	25	26	27	28	29	30	31	32	33	34	35
Final GuHCl (M):	2.6	2.5	2.4	2.3	2.2	2.1	2.0	1.9	1.8	1.7	1.6	1.5

Tube #	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**
Solution **A** (µL):	12	11	10	9	8	7	6	5	4	3	2	1
Solution **B** (µL):	36	37	38	39	40	41	42	43	44	45	46	47
Final GuHCl (M):	1.4	1.3	1.2	1.1	1.0	0.9	0.8	0.7	0.6	0.5	0.4	0.3

7. Mix well and incubate for at least 48 hours[1] at room temperature to ensure equilibrium.
8. After incubation, load 48 capillaries from the tubes and place them on positions 1 to 48 of the capillary tray according to the vial number. Place the magnetic lid to fix the capillaries.
9. Start a new session of the *PR.ChemControl* software.
10. Go to 'Plan' and prepare a run with the following settings:
    a. Start concentration: 5 M
    b. End concentration: 0.3 M
11. Start the measurement.
12. Use the 'Three-State' fit to analyze the data.

D4. nanoDSF Results

ΔG_1 = 28.0 kJ/mol | $c50_1$ = 2.3 M
ΔG_2 = 74.1 kJ/mol | $c50_2$ = 2.8 M



D5. Reference Results/Supporting Results

N/A

E. Contributors

Andreas Langer[2]

[1] Large biomolecules such as monoclonal antibodies often require a very long 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.
[2] NanoTemper Technologies GmbH, München, Germany | nanotempertech.com