Prometheus Protocol PR-P-018

Incremental Cycling of Trastuzumab

Thermal unfolding of a lot of proteins can be partly reversible as long as the temperature is not increased beyond a critical value that induces non-reversible changes in the protein structure. Finding this temperature requires a different heating program than the widely used linear temperature ramp that is commonly applied to measure T_m values. In this protocol, the temperature of non-reversible unfolding of Trastuzumab (Herceptin) is determined using the *Incremental Cycle* mode of the *PR.TimeControl* software. Herceptin is a monoclonal antibody used to treat breast cancer.

thermal cycling | refolding | non-reversibility curve

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

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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

N/A

A5. Stock Concentration/Stock Buffer

120 mg/mL | 825 µM

A6. Molecular Weight/Extinction Coefficient

145.5 kDa

A7. Dilution Buffer

Phosphate buffered saline (PBS, pH 7.4), 0.05% TWEEN® 20

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

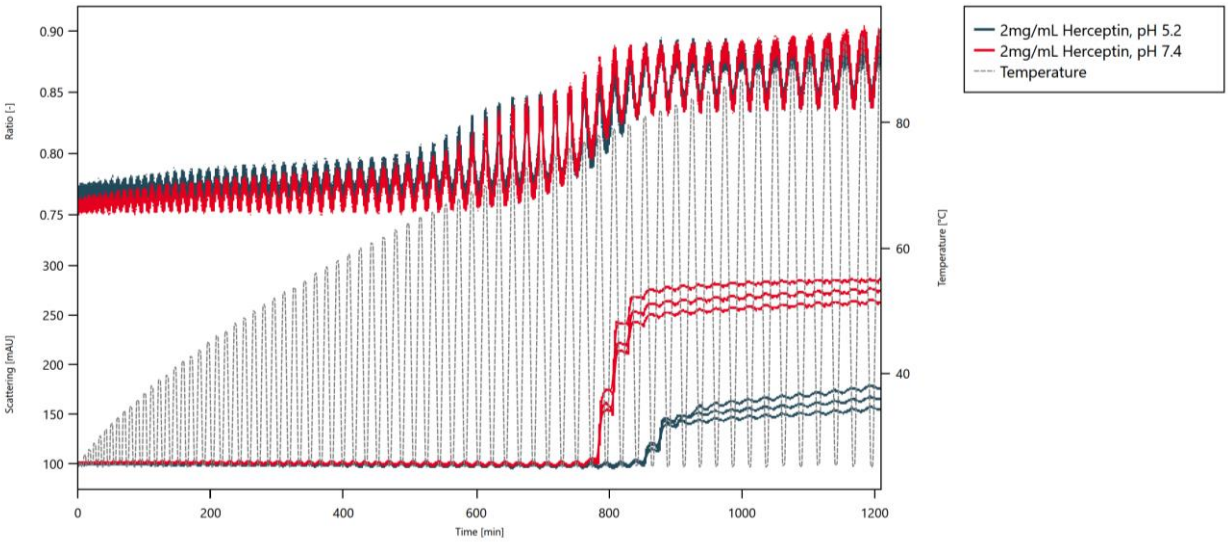
PR.TimeControl v1.0.2 (NanoTemper Technologies GmbH)
nanotempertech.com/prometheus-software

D3. nanoDSF Experiment

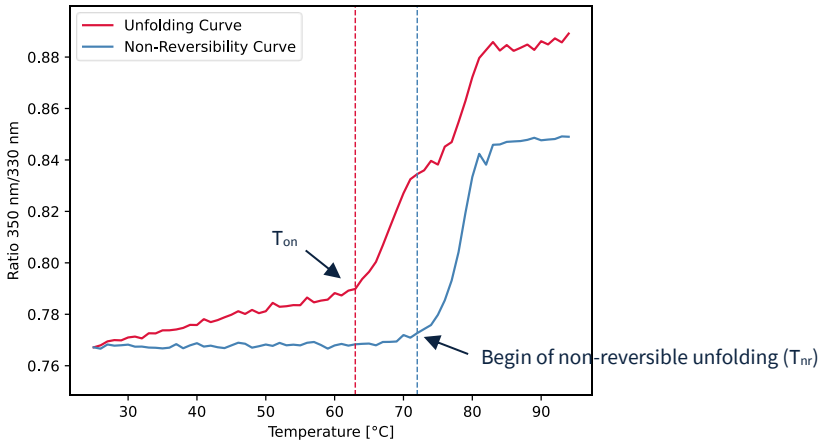
1. Prepare a 20 mg/mL solution of Trastuzumab in PBS buffer.
2. Mix 4 µL of the 20 mg/mL Trastuzumab solution with 36 µL of 20 mM Na-Acetate buffer, pH 5.2, to obtain 40 µL of a 2 mg/mL Trastuzumab pH 5.2 solution.
3. Then, mix 4 µL of the 20 mg/mL Trastuzumab solution with 36 µL of PBS buffer, pH 7.4, to obtain 40 µL of a 2 mg/mL Trastuzumab pH 7.4 solution.
4. Start a new session of the *PR.TimeControl* software.
5. Change the Thermostat Set Point to 25°C by using the touch display on the instrument and wait for the system to reach this temperature.
6. Go to 'Measurement Scan' and prepare a run with the following settings:
    a. Capillaries 1 – 6 selected
    b. Incremental Cycle
    c. First Temperature 25°C
    d. First Duration 2 min
    e. Temperature Increase 1°C
    f. Second Duration 2 min
    g. Measurement Cycles 70
    h. 5% excitation power
7. Load 3 capillaries from the 2 mg/mL Trastuzumab **pH 5.2** solution and place them on positions **1 – 3** of the Prometheus capillary tray.
8. Load 3 capillaries from the 2 mg/mL Trastuzumab **pH 7.4** solution and place them on positions **4 – 6** of the Prometheus capillary tray.
9. Place the magnetic lid to fix the capillary.
10. Start the measurement (duration: **~24 hours**).

D4. nanoDSF Results

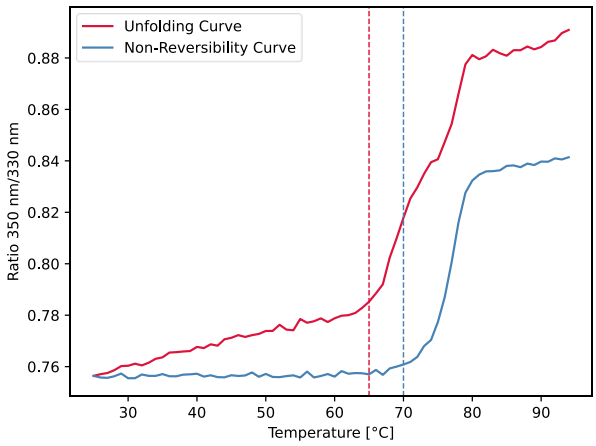
Data from *PR.TimeControl*:



Separating the data into ratio values at 'hot' (unfolding curve) and 'cold' (non-reversibility curve)[1]:



pH 5.2



pH 7.4

[1] See also Svilenov et al., Mol. Pharmaceutics 2020, 17, 7, 2638–2647 for more information on data analysis.

Trastuzumab	pH 5.2	pH 7.4
T_{on} (Onset temperature)	~63°C	~65°C
T_{nr} (Non-reversibility onset temperature)	~72°C	~70°C
T_{agg} (Aggregation onset temperature)	~81°C	~78°C

While Trastuzumab begins to unfold at a slightly lower temperature at pH 5.2 than at pH 7.4 (~63°C vs. ~65°C), the temperature of the first non-reversible unfolding is in fact higher (~72°C vs. ~70°C), which also correlates with the non-reversible aggregation information from the scattering signal (~81°C vs. ~78°C).

D5. Reference Results/Supporting Results

Svilenov et al., Mol. Pharmaceutics 2020, 17, 7, 2638−2647

E. Contributors

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