

Prometheus Protocol PR-P-004

Isothermal Denaturation of p38-alpha

Mitogen-activated protein kinase 14 (also called p38- α) is an enzyme that has been implicated in the regulation of many proinflammatory pathways. It is a drug target for many diseases. Kinetic analysis of the thermal stability of p38- α can be used for activation energy analysis and long-term stability prediction.

[thermal unfolding](#) | [unfolding kinetics](#) | [isothermal](#) | [Arrhenius equation](#) | [activation energy](#)

A1. Target/Fluorescent Molecule

Mitogen-activated protein kinase 14 (p38- α)

uniprot.org/uniprot/Q16539

A2. Molecule Class/Organism

p38 mitogen-activated protein kinases (MAP kinase)

Homo sapiens (Human)

A3. Sequence/Formula

MSQERPTFYR QELNKTIW^EEV PERYQNLSPV GSGAYGSVCA AFDTKTGLRV AVKKLSRPFQ SIIHAKRTYR ELRLKHKMKH
 ENVIGLLDVF TPARSLEEFN DVYLVTHLMG ADLNNIVKCQ KLTDDHVQFL IYQILRGLKY IHSADIIHRD LKPSNLAVNE
 DCELKILDFG LARHTDDEMT GYVATRWYRA PEIMLNW^MMHY NQTVDI^WSVG CIMAELLTGR TLFPGTDHID QLKLI^LRLVG
 TPGAELKKI SSESARNYIQ SLTQMPKMNF ANVFIGANPL AVDLLEKMLV LDSDKRITAA QALAHAYFAQ YHDPDDEPVA
 DPYDQSFESR DLLIDE^WKSL TYDEVISFVP PPLDQEEMES

A4. Purification Strategy/Source

Expressed in E. coli BL21, His₆-tagged

CreLux GmbH

A5. Stock Concentration/Stock Buffer

8.82 mg/mL | 197 μ M

25 mM HEPES, pH 7.4, 50 mM NaCl, 10 mM DTT, 1 mM EDTA

A6. Molecular Weight/Extinction Coefficient

44.7 kDa

50,100 M⁻¹cm⁻¹ (ϵ_{280})

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

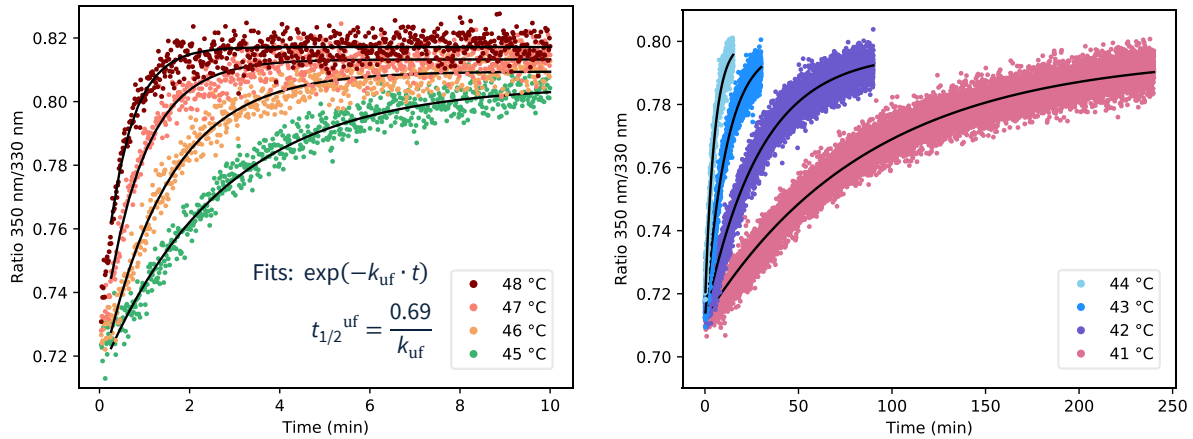
D3. nanoDSF Experiment

1. Add 195 μL of dilution buffer to 2 μL of 197 μM p38- α to obtain 197 μL of a 2 μM solution.
2. Start a new session of the *PR.TimeControl* software.
3. Change the Thermostat Set Point to 48°C by using the touch display on the instrument and wait for the system to reach this temperature.
4. Go to 'Measurement Scan' and prepare a run with the following settings:
 - a. Only capillary 1 selected
 - b. Isothermal
 - c. 48°C
 - d. 10 min
 - e. 20% excitation power
5. Load one capillary from the 2 μM p38- α solution and place it on position 1 of the Prometheus capillary tray.
6. Place the magnetic lid to fix the capillary.
7. Start the measurement.
8. After the measurement is finished, repeat steps 3 – 7 for temperatures of 47°C, 46°C and 45°C. Note that the temperature needs to be set on instrument touch display (step 3) **and** in the PR.TimeControl software (step 4c).
9. Repeat steps 3 – 7 for temperatures of 44°C, 43°C, 42°C and 41°C. Increase the measurement duration (step 4d) to 15 min, 30 min, 90 min and 240 min, respectively.

D4. nanoDSF Results

T (°C)	48	47	46	45	44	43	42	41
Measurement duration (min)	10	10	10	10	15	30	90	240
k_{uf} ($10^{-3} s^{-1}$)	30.7	18.6	10.8	6.0	3.2	1.4	0.56	0.19
$t_{1/2}^{uf}$ (min)	0.4	0.6	1.1	1.9	3.6	8.3	20.6	60.8

Kinetic data from *PR.TimeControl* (data points) and mono-exponential fits¹ (solid lines):



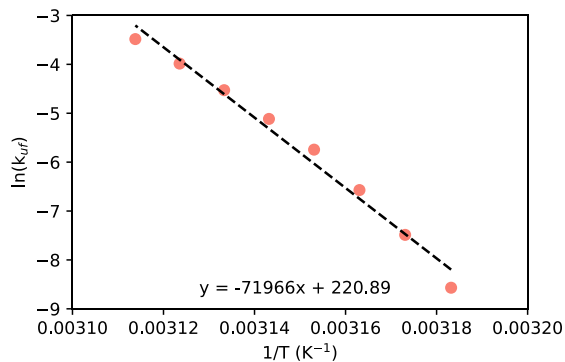
The temperature dependent (irreversible) unfolding rate is described by the Arrhenius equation²:

$$k_{uf} = A \cdot \exp\left(-\frac{E_a}{RT}\right) \tag{1}$$

where A is the frequency factor (s^{-1}) and E_a is the activation energy ($kJ mol^{-1}$). It follows:

$$\ln(k_{uf}) = \ln(A) - \frac{E_a}{RT} \tag{2}$$

Plotting of $\ln(k_{uf})$ vs. $1/T$ yields:



¹ Exponential fits of the data cannot be performed in the *PR.TimeControl* software but have to be done in a third-party software such as Microsoft Excel™.

² For an excellent overview, see [Qin et al., Annals of Biomedical Engineering 42 \(2014\) 2392–2404.](#)

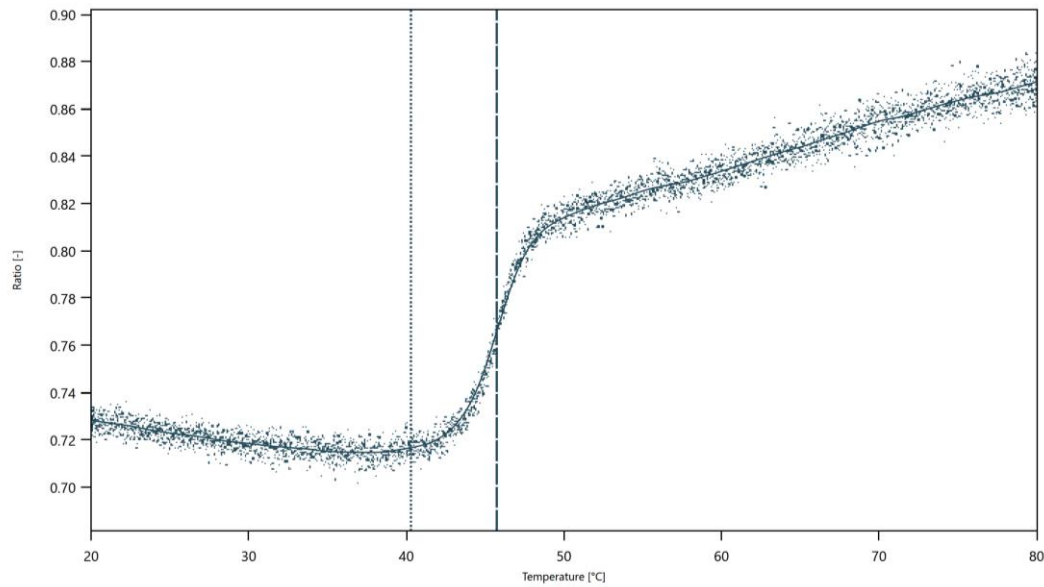
From linear fitting, one obtains:

$$\ln(A) = 220.89 \rightarrow A = 8.5 \cdot 10^{95} \text{ s}^{-1}$$

$$\frac{E_a}{R} = 71,966 \text{ K} \rightarrow E_a = 8.314 \frac{\text{J}}{\text{K} \cdot \text{mol}} \cdot 71,966 \text{ K} = 598 \text{ kJ mol}^{-1}$$

D5. Reference Results/Supporting Results

$T_m = 45.8^\circ\text{C}$ | $T_{on} = 40.3^\circ\text{C}$ nanoDSF (thermal unfolding measurements, $1^\circ\text{C}/\text{min}$)



E. Contributors

Andreas Langer³

³ NanoTemper Technologies GmbH, München, Germany | nanotempertech.com