Prometheus Protocol PR-P-008

Heating Rate Dependent 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. Thermal unfolding of p38-α at different heating rates can be used for activation energy analysis and long-term stability prediction.

thermal unfolding | heating rate | 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

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MSQERPTFYR QELNKTIWEV PERYQNLSPV GSGAYGSVCA AFDTKTGLRV AVKKLSRPFQ SIIHAKRTYR ELRLLKHMKH
ENVIGLLDVF TPARSLEEFN DVYLVTHLMG ADLNNIVKCQ KLTDDHVQFL IYQILRGLKY IHSADIIHRD LKPSNLAVNE
DCELKILDFG LARHTDDEMT GYVATRWYRA PEIMLNWMHY NQTVDIWSVG CIMAELLTGR TLFPGTDHID QLKLILRLVG
TPGAELLKKI SSESARNYIQ SLTQMPKMNF ANVFIGANPL AVDLLEKMLV LDSDKRITAA QALAHAYFAQ YHDPDDEPVA
DPYDQSFESR DLLIDEWKSL TYDEVISFVP PPLDQEEMES
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A4. Purification Strategy/Source

Expressed in E. coli BL21, His_6-tagged
Crelux GmbH

A5. Stock Concentration/Stock Buffer

8.82 mg/mL | 197 µM
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 $M^{-1}cm^{-1}$ (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

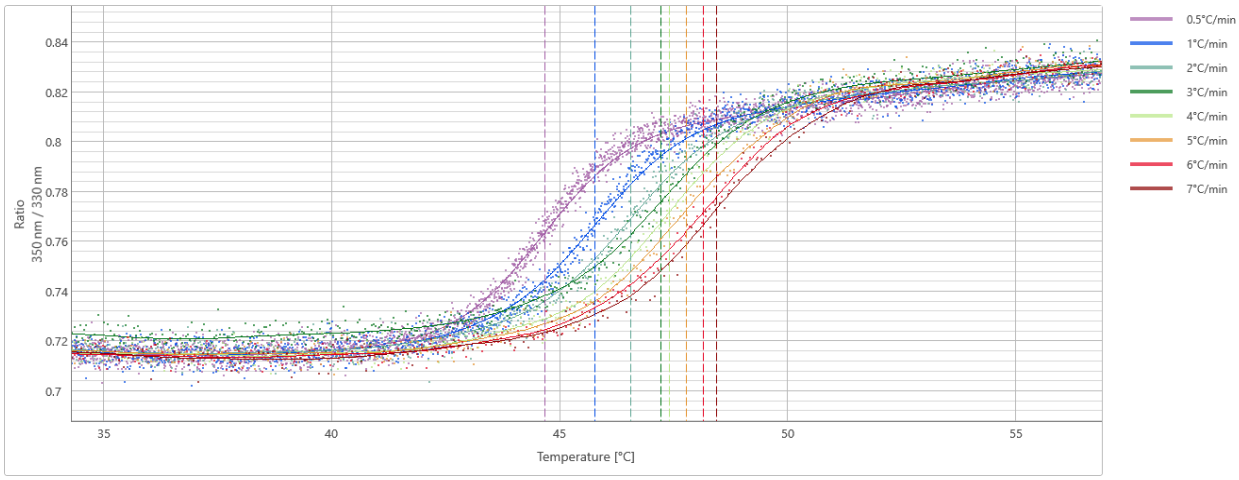
PR.ThermControl v2.1 | PR.StabilityAnalysis v1.1 (NanoTemper Technologies GmbH)
nanotempertech.com/prometheus-software

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.ThermControl* software.
3. Completely fill one capillary from this solution, place it on position 1 of the capillary tray and place the magnetic lid to fix the capillaries.
4. Go to 'Melting Scan' and prepare a run with the following settings:
    a. Only capillary 1 selected
    b. 0.5°C/min
    c. 20°C – 75°C
    d. 20% excitation power
5. Start the measurement.
6. After the measurement is finished, repeat steps 3 – 5 with heating rates from 1°C/min to 7°C/min.

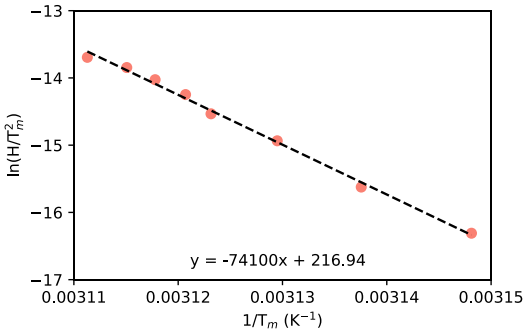
D4. nanoDSF Results

H (°C/min)	**0.5**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
H (K/s)	0.0083	0.0167	0.0333	0.0500	0.0667	0.0833	0.1000	0.1167
T_m (°C)[1]	44.50	45.57	46.39	47.04	47.29	47.59	47.87	48.26



[1] T_m values were obtained by thermodynamic fits in the PR.StabilityAnalysis software.

Plotting $\ln(H/T_{\mathrm{m}}^2)$ against $1/T_{\mathrm{m}}$ (Kissinger method) yields[2]:



$$\text{slope} = -74100\ \text{K}$$

Then, the activation energy of thermal denaturation E_a can be calculated as:

$$E_a = -\text{slope} \cdot \text{R} = -74100\ \text{K} \cdot 8.314\,\frac{\text{J}}{\text{K} \cdot \text{mol}} = 616\,\frac{\text{kJ}}{\text{mol}}$$

D5. Reference Results/Supporting Results

E_a = 598 kJ/mol Isothermal Denaturation
Prometheus Protocol PR-P-004

E. Contributors

Andreas Langer[3]

[2] For calculation of the natural logarithm, T_m needs to be in the units of 'K' and H in the units of 'K/s', **not** '°C/min'. See also Kissinger, Journal of Research of the National Bureau of Standards, 57 (1956) 217-221 for more information.

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

Appendix: Mathematical derivation of the Kissinger equation

The temperature dependent (irreversible) unfolding rate is described by the Arrhenius equation[4]:

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

where A is the frequency factor (s^{-1}) and E_a is the activation energy ($\mathrm{kJ/mol}$).

If y is the ratio of folded protein at temperature T, then y changes over time according to

$$\frac{\mathrm{d}y}{\mathrm{d}t} = -k_{\mathrm{uf}} \cdot y = -A \cdot \exp\left(-\frac{E_{\mathrm{a}}}{RT}\right) \cdot y \tag{2}$$

In a thermal unfolding measurement with constant heating rate, the temperature T is increasing linearly, i.e.

$$T = T(t) = T_0 + H \cdot t \quad \rightarrow \quad dT = H \cdot dt \tag{3}$$

where H is the heating rate and T_0 the starting temperature of the unfolding measurement. It follows

$$\frac{\mathrm{d}y}{\mathrm{d}T} = -\frac{A}{H} \cdot \exp\left(-\frac{E_{\mathrm{a}}}{RT}\right) \cdot y \tag{4}$$

The melting temperature T_{m} is the temperature at which $\mathrm{d}y/\mathrm{d}T$ is maximal, i.e.

$$\frac{\mathrm{d}}{\mathrm{d}T}\left(\frac{\mathrm{d}y}{\mathrm{d}T}\right) = \frac{\mathrm{d}}{\mathrm{d}T}\left(-\frac{A}{H} \cdot \exp\left(-\frac{E_{\mathrm{a}}}{RT}\right) \cdot y\right) = \frac{\mathrm{d}}{\mathrm{d}T}\left(\exp\left(-\frac{E_{\mathrm{a}}}{RT}\right) \cdot y\right) = 0 \tag{5}$$

Thus,

$$\frac{E_{\mathrm{a}}}{RT_{\mathrm{m}}^2} \cdot y + \frac{\mathrm{d}y}{\mathrm{d}T} = \frac{E_{\mathrm{a}}}{RT_{\mathrm{m}}^2} \cdot y - \frac{A}{H} \cdot \exp\left(-\frac{E_{\mathrm{a}}}{RT_{\mathrm{m}}}\right) \cdot y = 0 \tag{6}$$

$$\frac{H}{T_{\mathrm{m}}^2} = \frac{R}{E_{\mathrm{a}}} \cdot A \cdot \exp\left(-\frac{E_{\mathrm{a}}}{R} \cdot \frac{1}{T_{\mathrm{m}}}\right) \tag{7}$$

$$\ln\left(\frac{H}{T_{\mathrm{m}}^2}\right) = \ln\left(\frac{R}{E_{\mathrm{a}}} \cdot A\right) - \frac{E_{\mathrm{a}}}{R} \cdot \frac{1}{T_{\mathrm{m}}} \tag{8}$$

$$\frac{\mathrm{d}\ln(H/T_{\mathrm{m}}^2)}{\mathrm{d}\,1/T_{\mathrm{m}}} = -\frac{E_{\mathrm{a}}}{R} \tag{9}$$

Hence, plotting $\ln(H/T_{\mathrm{m}}^2)$ against $1/T_{\mathrm{m}}$ will yield a line with the slope $-E_{\mathrm{a}}/R$.

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