Prometheus Protocol PR-P-007

Thermal Unfolding of MHC Class I Molecules

Major Histocompatibility Complex class I (MHC class I) molecules present variable and non-self antigenic peptides to $CD8^+$ T cells. In this way, they play a very significant role in our body’s immune defense mechanisms against pathogens and tumors. MHC class I molecules are used to generate MHC tetramers that have wide applications in T cell staining, isolation, and activation. In immunotherapy, MHC tetramers are used to trace T cells in patients' blood. Hence, these MHC class I molecules are popular topics of research. Biochemical characterization of these proteins involves studies on structural stability, peptide binding affinity, pH stability, and storage conditions. The thermal denaturation method can be used to answer all these parameters.

thermal unfolding | T_m | MHC class I

A1. Target/Fluorescent Molecule

HLA class I histocompatibility antigen, HLA-A*02:01[1] or A2
uniprot.org/uniprot/P01892

A2. Molecule Class/Organism

Immune receptors
Homo sapiens (Human)

A3. Sequence/Formula

A2 Heavy Chain

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GSHSMRYFFT SVSRPGRGEP RFIAVGYVDD TQFVRFDSDA ASQRMEPRAP WIEQEGPEYW DGETRKVKAH SQTHRVDLGT
LRGYYNQSEA GSHTVQRMYG CDVGSDWRFL RGYHQYAYDG KDYIALKEDL RSWTAADMAA QTTKHKWEAA HVAEQLRAYL
EGTCVEWLRR YLENGKETLQ RTDAPKTHMT HHAVSDHEAT LRCWALSFYP AEITLTWQRD GEDQTQDTEL VETRPAGDGT
FQKWAAVVVP SGQEQRYTCH VQHEGLPKPL TLRW
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Beta-2 microglobulin Light Chain

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MSRSVALAVL ALLSLSGLEA IQRTPKIQVY SRHPAENGKS NFLNCYVSGF HPSDIEVDLL KNGERIEKVE HSDLSFSKDW
SFYLLYYTEF TPTEKDEYAC RVNHVTLSQP KIVKWDRDM
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A4. Purification Strategy/Source

Size exclusion chromatography

A5. Stock Concentration/Stock Buffer

1 mg/mL | 22 µM
50 mM Tris-HCl, pH 8, 150 mM NaCl, 10% Glycerol

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[1] HLA-A*02:01 is most abundant among the European population.

A6. Molecular Weight/Extinction Coefficient

45.4 kDa
93,880 $M^{-1}cm^{-1}$ (ε_{280})

A7. Dilution Buffer

50 mM Tris-HCl, pH 7.8, 150 mM NaCl, 10 mM $MgCl_2$, 0.05% TWEEN 20

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.ThermControl v2.1 (NanoTemper Technologies GmbH)
nanotempertech.com/prometheus-software

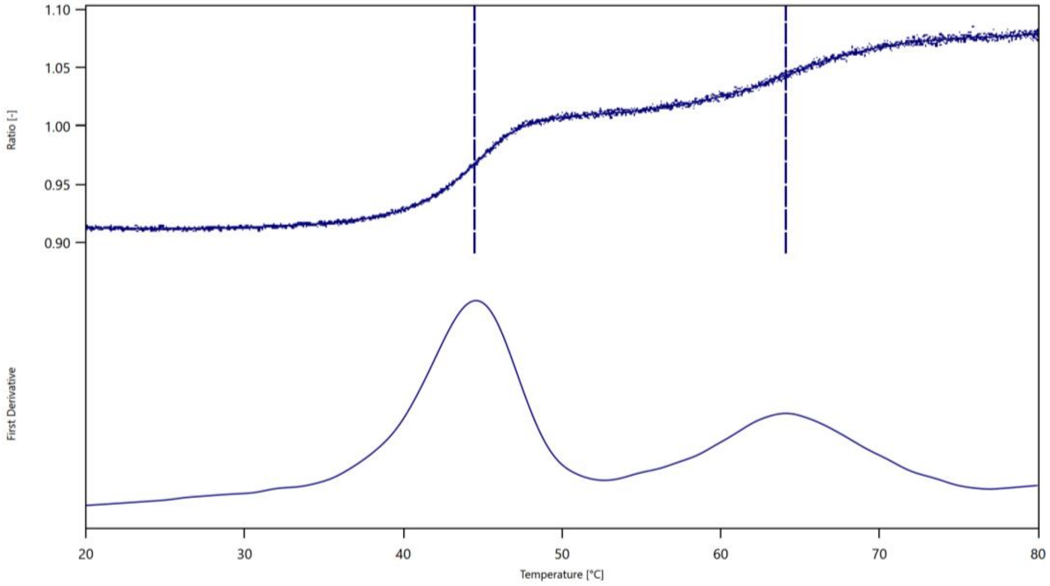
D3. nanoDSF Experiment

1. Re-suspend folded and purified HLA-A*02:01/peptide complexes in 50 µL dilution buffer to obtain a 100 µg/ml solution.
2. Load capillaries with samples and place them on the capillary tray.
3. Place the magnetic lid to fix the capillaries.
4. Start a new session of the *PR.ThermControl* software.
5. Go to ‘Melting Scan’ and prepare a run with the following settings:
    a. Select capillaries with samples.
    b. 1.0°C/min
    c. 20°C – 80°C
    d. 80% excitation power
6. Start the measurement.

D4. nanoDSF Results

Case 1: MHC class I folded with lower affinity peptide: HLA-A*02:01/NGVPMVATV[2]

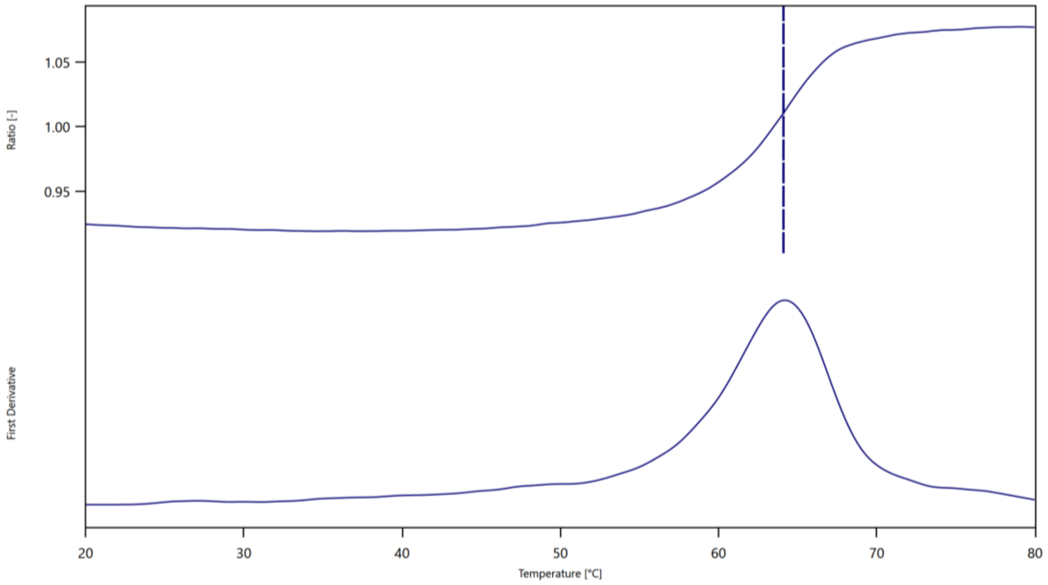
T_m (HC) = 44.5°C | T_m (LC) = 64.1°C



Two inflection points (T_m) can be observed. The first one at 44.5°C corresponds to the unfolding of the heavy chain (HC), the second one at 64.1°C corresponds to the unfolding of the light chain β_2m (LC).

Case 2: MHC class I folded with high affinity peptide: HLA-A*02:01/NLVPMVATV (CMV pp65)

T_m (HC, LC) = 64.1°C



Only one inflection point (T_m) can be observed. It corresponds to the unfolding of both, heavy chain (HC) and light chain β_2m (LC).Both chains denature at the same temperature and the peaks overlap.

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[2] Modified version of high affinity peptide NLVPMVATV (CMV pp65) where the second amino acid in the sequence is replaced with a Glycine (G).

D5. Reference Results/Supporting Results

$T_m = 63.9°C$	Differential Scanning Fluorometry (DSF) Hassan et al., JBC 290(5), 2015, 2593-2603
$T_m = 56.0°C$	Thermal denaturation by tryptophan fluorescence (TDTF) Saini et al., PNAS 110(38), 2013, 15383-15388

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