Prometheus Protocol PR-P-014

Stabilizing Effects of Compatible Solutes

Compatible solutes or osmoprotectants form a class of highly abundant organic molecules found in extremophilic bacteria. These molecules protect cells from osmotic and thermal stress by stabilizing protein and membrane structures through extensive hydration. Osmoprotectants typically accumulate in extremely high concentrations in the cell without showing any sign of toxicity or interference with metabolic pathways, making them a promising candidate for stabilization of protein-based therapeutics.

compatible solutes | osmoprotectants | thermal unfolding | conformational stability | colloidal stability | aggregation | T_m | T_{on} | T_{agg}

A1. Target/Fluorescent Molecule

Herceptin (Trastuzumab)

A2. Molecule Class/Organism

Monoclonal antibodies

A3. Sequence/Formula

Heavy chain

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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
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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
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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, 10 mM phosphate pH 7.4, 137 mM NaCl, 2.7 mM KCl)

D1. nanoDSF System/Capillaries

Prometheus NT.48 (NanoTemper Technologies GmbH)
Prometheus Aggregation Detection Optics (PR-AGO, NanoTemper Technologies GmbH)
Standard Capillaries Prometheus NT.48 nanoDSF Grade (PR-C002, NanoTemper Technologies GmbH)

D2. nanoDSF Software

PR.ThermControl v2.1 | PR.Stability Analysis v1.0.2 (NanoTemper Technologies GmbH)
nanotempertech.com/prometheus-software

D3. nanoDSF Experiment

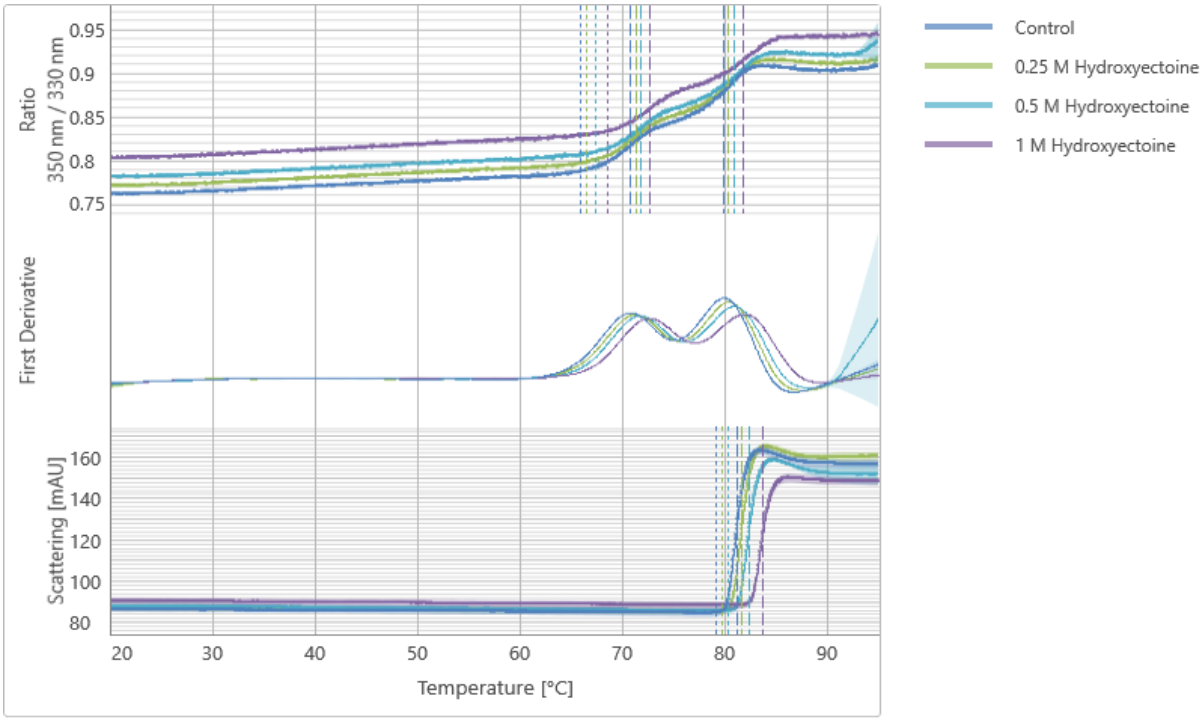
1. Dilute 5 µL of Herceptin with 55 µL of dilution buffer to obtain a 10 mg/mL solution.
2. Prepare a 4 M stock of hydroxyectoine by dissolving 64 mg hydroxyectoine in 100 µL of dilution buffer.
3. Prepare the following predilutions:

	Hydroxyectoine stock [µL]	**Assay buffer [µL]**	**Total [µL]**
Control	0	45	45
0.25 M Hydroxyectoine	3.1	41.9	45
0.5 M Hydroxyectoine	6.2	38.8	45
1 M Hydroxyectoine	12.5	32.5	45

4. Add 5 µL of the 10 mg/mL Herceptin solution to every predilution from step 3 to obtain a final concentration of 1 mg/mL Herceptin in every sample.
5. Completely fill four capillaries from every solution, place them on positions 1 – 16 of the capillary tray and place the magnetic lid to fix the capillaries.
6. Start a new session of the *PR.ThermControl* software.
7. Go to 'Melting Scan' and prepare a run with the following settings:
    a. Capillaries 1 – 16 selected
    b. 1.0°C/min
    c. 20°C – 95°C
    d. 25% excitation power
8. Start the measurement.

D4. nanoDSF Results

	T_{on} (° C)	$T_{m,1}$ (° C)	$T_{m,2}$ (° C)	T_{agg} (° C)
Control	65.90 ± 0.29	70.86 ± 0.08	79.88 ± 0.06	79.24 ± 0.06
0.25 M Hydroxyectoine	66.50 ± 0.11	71.34 ± 0.07	80.35 ± 0.05	79.71 ± 0.01
0.5 M Hydroxyectoine	67.40 ± 0.38	71.84 ± 0.06	80.88 ± 0.04	80.38 ± 0.01
1 M Hydroxyectoine	68.56 ± 0.11	72.72 ± 0.08	81.84 ± 0.08	81.70 ± 0.09



D5. Reference Results/Supporting Results

N/A

E. Contributors

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