

QuantStudio 6/7: CMI Getting Started Guide to Differential Scanning Fluorimetry

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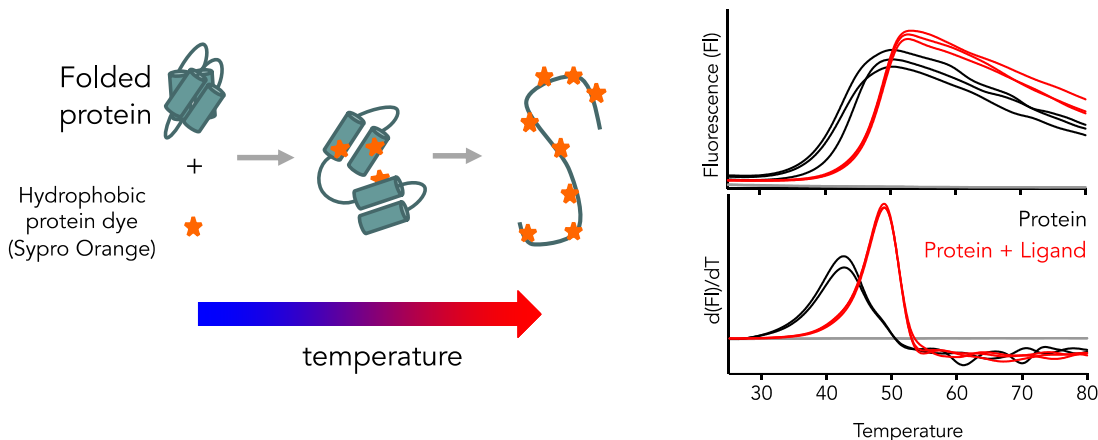
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Introduction

Differential Scanning Fluorimetry (DSF) uses a real-time PCR instrument to monitor thermally induced protein denaturation by measuring changes in fluorescence of a dye that binds preferentially to unfolded protein (such as Sypro Orange, which binds to hydrophobic regions of proteins exposed by unfolding). This experiment is also known as a [Protein Thermal Shift Assay](#), because shifts in the apparent melting temperature can be measured upon the addition of stabilizing or destabilizing binding partners or buffer components.



Instrument Overview

The CMI has a modified [Quant Studio 6/7](#) from Life Technologies for conventional DSF. It is a hybrid between a QuantStudio 6 and 7, with decoupled excitation and emission filters.

Applications

- Unfolding temperature (T_m)
- High-throughput buffer optimization
- Small molecule binding screen

Key Features

- Rapid data collection (~30 min/96-well plate)
- Low sample consumption (typically 1-5 μg /well)
- Proprietary LifeTechnologies Protein Thermal Shift Analysis Software

Required Supplies

- 96-well FAST-block optical plate, e.g. LifeTechnologies MicroAmp FAST optical 96-well reaction plate, 0.1 mL, 4346907
- Optical adhesive film, e.g. LifeTechnologies MicroAmp Optical Adhesive Film, 4360954
- DSF compatible dye, e.g. LifeTechnologies Protein Thermal Shift Dye Kit, 4461146 (Sypro Orange)

Sample Preparation

Assay Buffers

- Differences in buffering agent, salt, additive composition, concentrations of components, or pH can result in variability in protein thermal stability.
 - DSF is a good tool for comparing the effect of different buffers on protein stability
- When comparing thermal stability of mutant proteins or protein/ligand complexes, it is important to *match buffers between samples*.
- Hydrophobic buffers, including those with detergent, will bind to Sypro Orange and lead to high background staining.
- Assay buffer should never include protein, as any protein will bind to Sypro Orange and the unfolding of the buffer protein will likely drown out the signal of your protein of interest. This includes BSA, which has very high background staining with Sypro Orange.

Fluorescent Dye

- Concentration of fluorescent dye should be optimized for best results
- Sypro Orange, a hydrophobic dye that binds protein upon denaturation, is the most common DSF dye. Hydrophobic dyes are incompatible with detergent and other hydrophobic buffer components.
- Sypro Orange dyes are sold as either 1000X Protein Thermal Shift dye kit or 5000X Sypro Orange Protein Stain. *These reagents have the same concentration*, and both be used dilutions of 1/200 – 1/1000:
 - 1000X Protein Thermal Shift dye kit, final concentration 0.2X-5X (1X most common)
 - 5000X Sypro Orange Protein Stain, final concentration 1X-20X (5X most common)
- Cysteine-reactive dyes, such as Bodipy-cysteine, which can be used with membrane proteins in detergent if the protein has a buried free cysteine.

Protein Samples

- Concentration of protein should be optimized for best results
- Protein: 0.05-10 µg/well (1-5 µg)
- Total volume: 10-30 µL/well (20 µl)

Getting Started

Resources

Additional resources are available at the instrument, including control and analysis software.

Experimental Design Tips

- Perform 3-4 replicates
- Perform a No-Protein Control (NPC) with buffer and fluorescent dye to test for reaction of buffer components with the dye and/or background fluorescence

General Care and Maintenance

- Keep the instrument door (on the right side) free of obstruction.
- If the control software won't connect to the instrument, restart the software. If the control software still won't connect, cycle the power on both the instrument and the computer.

Startup

1. Book time on the PPMS calendar before you start.
2. Login to the computer using your PPMS credentials (eCommons ID and password).
3. Set up the DSF plate
 - a. Mix protein, buffer and fluorescent dye (added last).
 - b. Load 96-well FAST block optical plate. Include replicates and No-Protein Controls.
 - c. Cover the plate with optical film
4. Tap the touchscreen to wake the instrument from sleep by pressing the power button.
5. Open the instrument door.
6. Place the plate in the instrument and close the door.

Data Collection

1. Open **Quant Studio Real-Time PCR Software**.
2. On the Setup tab, select Template
 - a. Open the file Standard Protein Thermal Shift template_x1m3.edt (located on the desktop).
3. Setup Tab.
 - a. Experiment Properties.

i) Experiment name:	provide a name
ii) Instrument:	Quant Studio 7
iii) Block:	FAST 96
iv) Type:	Melt Curve
v) Reagents:	Other
vi) Properties:	Standard
 - b. Define.

i) Targets:	Target 1
(1) Reporter:	ROX
(2) Quencher:	NONE
ii) Passive Reference:	NONE
 - c. Assign
 - i) Select entire plate (even if only partially filled)
 - (1) this will avoid losing data from human error in well selection
 - (2) The entire plate is read no matter which wells are selected
 - ii) Do not label wells at this time (labels are not transferred to the analysis software).
 - d. Run Method.
 - i) Set reaction volume: 20 μ L.
 - ii) Graphical View (set ramp parameters)
 - (1) select continuous collection.
 - (2) 25 °C 2 min, 1.6° C/s.
 - (3) 0.05 °C/s ramp.

- (4) 99 °C 2 min (approximate run time 30 min).
- e. Optical Filters: select x1-m3 for **melt curve filter**; deselect others (x4-m4 is the standard ROX set, which works but has a lower S/N).
- 4. Save experiment to your folder.
- 5. Run Tab: click Start Run and select the instrument number from the pulldown
- 6. (x4-m4 is the standard ROX set. If you select x1-m3, then you will receive an error message stating that the ROX filter set has not been selected... run it anyway).
- 7. After data collection, **you must press the Analyze button before proceeding to data analysis.**
- 8. Save experiment.

Data Analysis

The Protein Thermal Shift Software is proprietary and can only be used to analyze data collected on Life Technologies instruments. It can be used to merge and compare data, and fit thermal inflection points using a first-derivative model or Boltzmann model.

1. Open **Protein Thermal Shift Software**.
2. Create Study
3. Setup Tab
 - a. Study Properties.
 - i) Define study name.
 - ii) Select instrument type: Quant Studio 6/7.
 - b. Conditions. Add conditions to define well content, eg:
 - i) Protein
 - ii) Ligand
 - iii) Buffer
 - iv) Analysis Group (each analysis group can have only one reference sample type)
 - c. Experiment Files
 - i) Import data saved from QuantStudio Real-Time PCR software.
 - ii) Assign wells.
 - (1) Task
 - (a) Samples: protein and dye-containing regular samples.
 - (b) Reference: the sample to which all other samples in the analysis group are compared, and from which ΔT_m is calculated.
 - (c) NPC (no protein control) is used to examine background buffer interference with dye fluorescence (should be minimal).
 - (2) Analysis group –all samples you wish to be compared.
 - (3) Well components.
4. Analysis Tab.
 - a. Well Results to examine traces and descriptions, then process data with **Analyze** button.
 - b. Replicate Results and examine replicates as a group.
5. Export Raw or processed data.

Shutdown

1. Remove your plate from the instrument.
2. Clean up in and around the instrument.
3. Close the control and analysis software.
4. **Logoff from PPMS!**

Book time and Report Problems through the PPMS system: <https://ppms.us/hms-cmi>

- rates are based on booked and real-time usage.

Contact cmi@hms.harvard.edu with questions.

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