Thermal shifts (DSF) using the Bio-Rad CFX Connect

Preparing the plate:

Prepare your plate using the supplied plates and plate seals in the cupboard above the instrument. Recommended total well volume = 25 µL (5 µL min – 50 µL max; better results are achieved with ≥ 20 µL).

Typical protein concentration is ~ 5 µM (> 1 µM for a 20 kDa protein).

Suggested Sypro Orange dilution 1:~2000, e.g. prepare a fresh 1:400 stock of dye (usually supplied as a 5000X stock in DMSO) in buffer, and add 5 µL to each well.

Pipette with care to avoid bubbles, or use a plate-spinner prior to the run.

Press the seal down thoroughly to avoid evaporation losses.

Always load duplicate/triplicate wells.

Optimising protein and Sypro Orange concentrations:

Before running your experiment with ligands or in different buffers, it is advisable to optimize the concentrations of both your target protein and Sypro Orange as signal varies for each protein and excess dye can affect protein denaturation.

This optimization is easiest done by testing 4-5 protein concentrations against 4-5 Sypro Orange concentrations, like in the table below for 2-fold dilution series. Optimal molar concentration will depend also on the molecular weight of the protein, so use the table below as a starting point for 30-40 kDa protein. Adjust the range up for smaller proteins and down for larger proteins.

Protein:	**20 µM**	**10 µM**	**5 µM**	**2.5 µM**	**1.25 µM**
20x Sypro Orange					
10x Sypro Orange					
5x Sypro Orange					
2.5x Sypro Orange					
1.25x Sypro Orange					

Run a normal thermal denaturation program and identify the condition where you achieve nice curves with good signal using minimal protein and Sypro Orange.

Katherine Stott, 6th January 2017
Optimisation added by Marko Hyvönen, 13th March 2023