Thermofluor Assay using SYPRO Orange
Thibodeau Lab

Required reagents and equipment:
SYPRO orange, 5000X concentrate in DMSO (ThermoFisher Scientific)
Plates for QPCR machine and optical sealing film (multiple manufacturers)

Reaction setup:
1. Dilute 5000X SYPRO concentrate to 200X working stock. This dilution can be made in your protein buffer and should be done immediately before the experiment.
2. Dilute concentrated protein to 2-10 μM in stock buffer or buffer supplemented with additive to 45 μl and store on ice.
3. Add 5 μl of the SYPRO working stock to the reaction mix for a total volume of 50 μl. Store on ice.
4. Aliquot into 96 or 384 well plates and seal with optical film.

Setup and initialize QPCR machine – will vary by manufacturer:
1. ROX dye settings will provide the spectral parameters necessary for SYPRO detection.
2. Temperature ramp rates and starting points may be varied depending on protein stability. Note that protein concentration and ramp rate may influence the apparent Tm assessed using this method.
3. Pre-cool sample chamber (if an available option) and read plate.

Data Analysis:
Most machines will provide display options for both raw data and derivatives of the data curves. Both can be used to identify the transition midpoint. Pre-transition fluorescence, both intensity and trends, should be carefully considered as they may reflect changes in protein conformation, folding, and stability. High initial fluorescence, in particular, should be considered as it may reflect improperly folded, misfolded or unfolded protein in the initial sample.

References:
Lavinder et al. High-throughput thermal scanning: a general, rapid dye-binding thermal shift screen for protein engineering. J. Am Chem. Soc. 2009: 3794-3795.
Ericsson et al. Thermofluor-based high-throughput stability optimization of proteins for structural studies. Analytical Biochemistry 2006: 289-298.