The thermal stability of a protein formulation depends not only on the intrinsic properties of the protein itself but also on buffer composition and the concentration at which the protein is formulated. With the DynaPro® Plate Reader, a variety of conditions can be dispensed into 96-, 384-, or 1536-well plate formats to quantify protein stability as a function of temperature, pH, concentration, and excipient profile.

In this experiment, a monoclonal antibody was prepared in bis-tris-propane buffer (50 mM) at pH 8.5 and pH 9.5 and concentrations of 0.47-15 mg/mL to identify the thermal stability of the protein at twelve different conditions. For each condition, 20 µL of solution was loaded in triplicate into a 384-well microtiter plate, and each well was covered in paraffin oil to prevent evaporation. The hydrodynamic radius of the antibody in each well was measured as the temperature of the entire plate was increased from 25°C to 85°C, at a rate of 0.1°C/min.

At pH 8.5, this protein clearly aggregates into large complexes as the temperature is increased, with \( r_h \) at 75°C ranging from 80 to 800 nm (Figure 1). Onset analysis in the Dynamics® software was used to calculate the temperature at which \( r_h \) begins to change significantly. At all concentrations, the onset temperature was relatively constant at 55.9±0.9°C, and \( r_h \) at the onset temperature is 5.3±0.2 nm (Figure 1).

In contrast, at pH 9.5, the hydrodynamic radius exhibits a shift from \( r_h = 5.4±0.4 \) to a second higher, but stable, value for temperatures above 65°C. This behavior is typical of a protein unfolding but not aggregating (Figure 2).

Dynamics® was used to fit a sigmoid relationship for each concentration to yield a temperature and radius at the midpoint of the sigmoidal curve (Figure 2, inset). Here, we observed a significant concentration-dependence in the midpoint temperature and radius. In particular, the radius at the midpoint temperature increased ~30%, and the final radius increased ~50% as a function of concentration. Although the high order aggregation seen at pH 8.5 is prevented at pH 9.5, the data from these sigmoid fits suggest that the protein may still be prone to oligomerization or be otherwise “unstable” at high concentrations.

Thus, the DynaPro® Plate Reader successfully enabled the measurement of thirty-six different samples as a function of temperature. This multiplexed approach can be easily extended to a variety of other formulation conditions for rapid characterization of protein behavior without excessive labor requirements.