

## UV/Vis Spectroscopy

*This application note demonstrates the ability of the PerkinElmer LAMBDA 365+ with Peltier Accessory and UVWinLab to monitor the unfolding and folding of a protein as the temperature is increased and decreased.*

## Monitoring of Protein Unfolding and Folding with UV/Vis Spectroscopy

### Introduction

$\alpha$ -chymotrypsinogen is a precursor, or zymogen, of chymotrypsin. Chymotrypsin fulfills the role of breaking down proteins into smaller peptides in the digestive tract. It also displays an interesting property in that, when it unfolds due to thermal denaturation, it is able to refold when cooled back to room temperature.

### Experimental

A 0.5 mg/mL solution of  $\alpha$ -chymotrypsinogen in 10 mM NaCl buffer (made to pH 3.0 with 1M HCl). This was measured using the PerkinElmer LAMBDA<sup>®</sup> 365+ with Peltier accessory. In order to determine the correct wavelength for monitoring the stage of denaturation, two samples of the solution were measured between 200-500 nm at 25°C and 85°C. The two resulting spectra were subtracted to determine the wavelength showing the most variation. This wavelength was used to monitor denaturation over a range of temperatures. The temperature ramping conditions used are shown in Table 1.

Table 1: Temperature ramping conditions used for the analysis of chymotrypsinogen.

| Temperature Range (°C) | Ramp Rate (°C/min) |
|------------------------|--------------------|
| 25-45                  | 5                  |
| 45-65                  | 1                  |
| 65-85                  | 5                  |

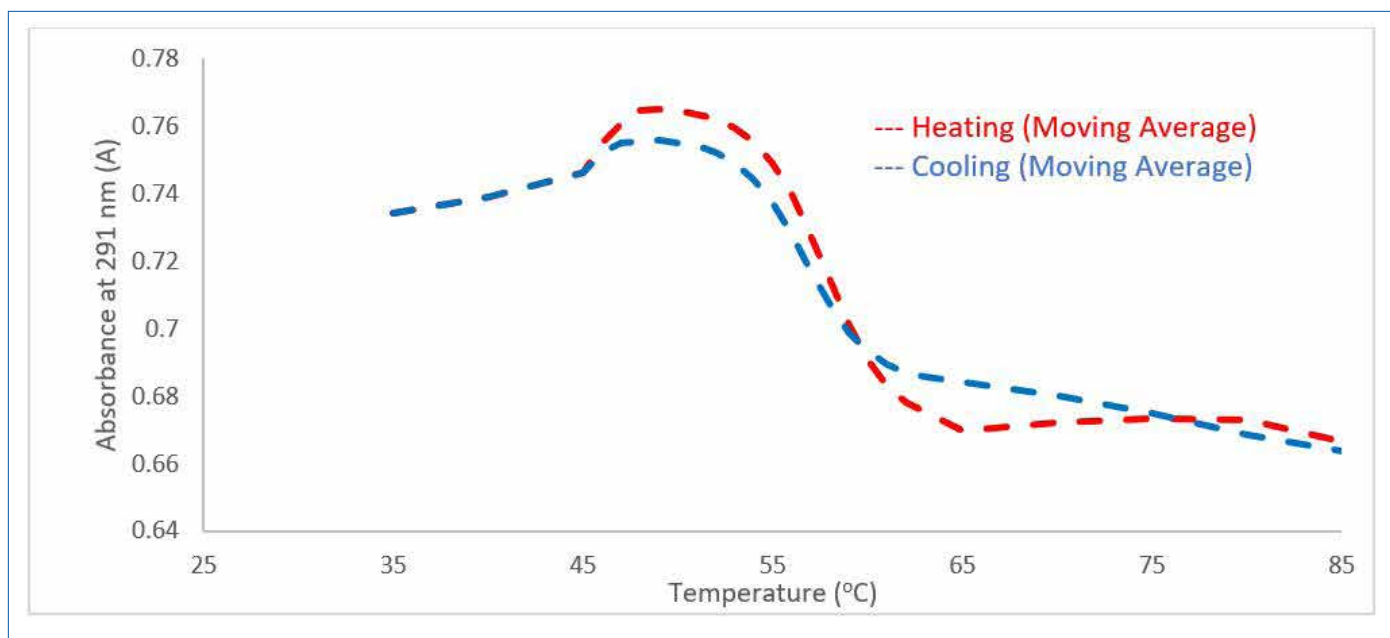


Figure 1: Heating/Cooling results from  $\alpha$ -chymotrypsinogen.

## Results and Discussion

PerkinElmer UVWinLab™ allows for software control of a Peltier accessory, including the option to use a multiple cell changer. UVWinLab also provides real time temperature vs absorbance data. The results from the heating and cooling of chymotrypsinogen are shown in Figure 1.

## Summary

UV/Vis spectroscopy with a Peltier accessory can be used to monitor changes in biological and biopharmaceutical samples with temperature. UVWinLab allows for full software control of the accessory and easy analysis of data.

## Reference

1. S. T. Freer, J. Kraut, J. D. Robertus, H. T. Wright, N. H. Xuong, *Biochemistry*, 1970, 9, 1997-2009