# Thermal denaturation of protein (by circular dichroism)

This protocol is used to determine the stability of protein. The thermal denaturation of protein can be monitored by circular dichroism spectrum. Usually, one wavelength (we use 220nm for our helical protein) is chosen to monitor.

## Protein sample preparation

- Phosphate buffer is good(Tris buffer is not appropriate for thermal denaturation, and high NaCl concentration can be a problem )
- Protein sample need to be at least 250uL for a 1mm CD cuvette and the concentration could be 2-50uM.(We use 8uM)

## Parameters for thermal denaturation

- ✧ 220nm
- ✧ 10-60°C
- ✧ 1 °C each step
- ✧ Equilibrium time: 30sec
- ✧ Heating rate: 2 °C/min
- ✧ Signal averaging time: 30sec
- ✧ 1nm bandwith

## Data analysis

Fit into two state unfolding model:

$$Y = \frac{(y_n + m_n T) + (y_d + m_d T)\exp(\Delta H_m / R(1/T_m - 1/T))}{1 + \exp(\Delta H_m / R(1/T_m - 1/T))}$$

$Y$:measured ellipticity
$\Delta Hm$: enthalpy at the unfolding transition
$T_m$: melting temperature
$T$: temperaturein Kelvin
$R$: universal gas constant
$m_n$: slope of the pretransition baseline
$y_n$ :intercept of pretransition baseline
$m_d$: slope of the post-transition baseline
$y_d$: intercept of the post-transition baseline

Reference:

1. EK Koepf, HM Petrassi, M Sudol and JW Kelly (1998) *Protein Sci*. **8**: 841-853

2. http://structbio.vanderbilt.edu/chazin/wisdom/labpro/CDthermal_melt.html