

## Supporting Information

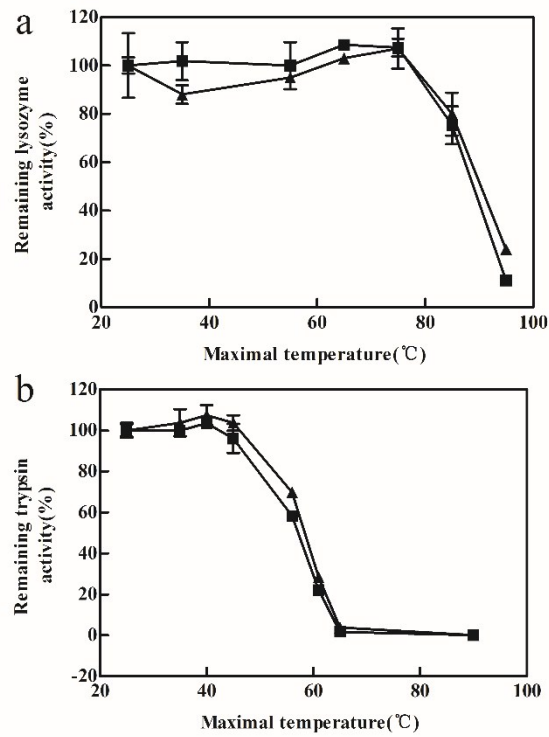
# **Measurement of the onset temperature of irreversible inactivation of proteins using FITC as a fluorescent reporter**

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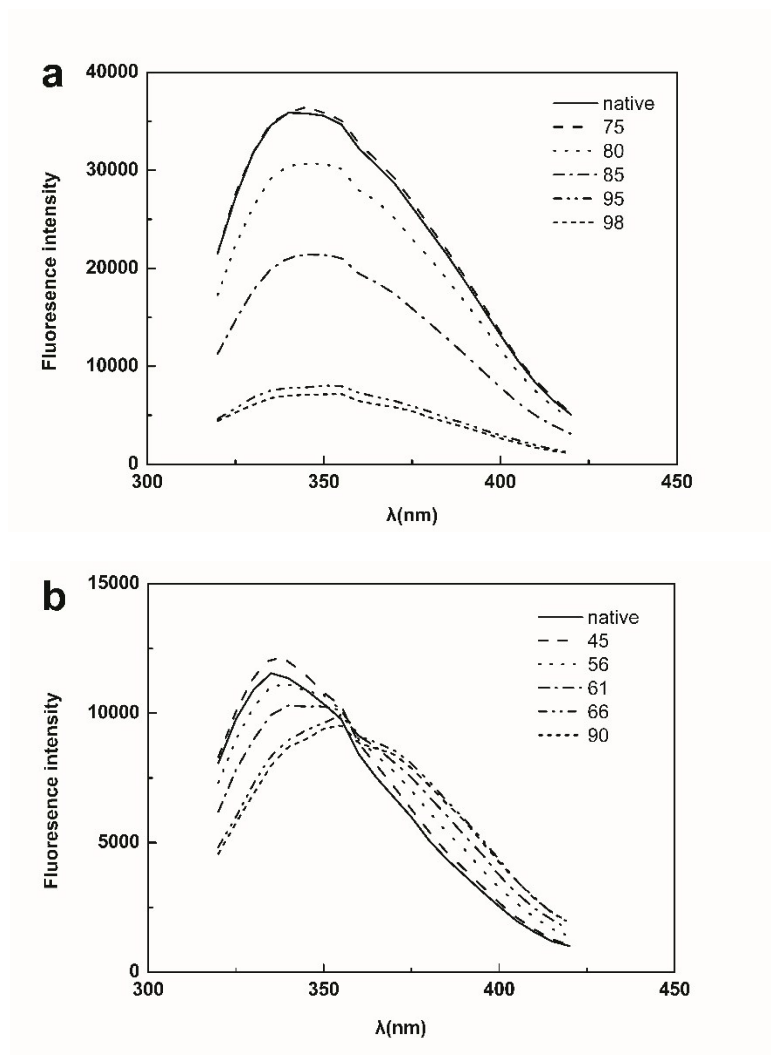
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**Figure S1.** (a) The dependence of the remaining activity of the FITC-labeled lysozyme (■) and the unlabeled lysozyme (▲) after a heating and cooling cycle to the  $T_{\max}$ . Lysozyme, 1.1mg/mL in 50mM potassium phosphate buffer (pH 7.0), was first heated to a  $T_{\max}$  and then cooled to room temperature at 1°C/30s. (b) The remaining activity of FITC-labeled trypsin (■) and unlabeled trypsin (▲) after a heating and cooling cycle to the  $T_{\max}$ . Trypsin, 0.12mg/mL in 50mM Tris-HCL (pH 9.0, containing 20mM CaCl<sub>2</sub>), was firstly heated to a  $T_{\max}$  and then cooled to room temperature at 1°C /30s.



**Figure S2.** Intrinsic tryptophan fluorescence emission spectra of (a) lysozyme and (b) trypsin after heating to different  $T_{\max}$  and cooling to the room temperature. Excitation wavelength: 295nm. Slit for emission: 5nm. Other conditions are as described in Figure 2.