

# Correlated Parameter Fit of Arrhenius Model for Thermal Denaturation of Proteins and Cells

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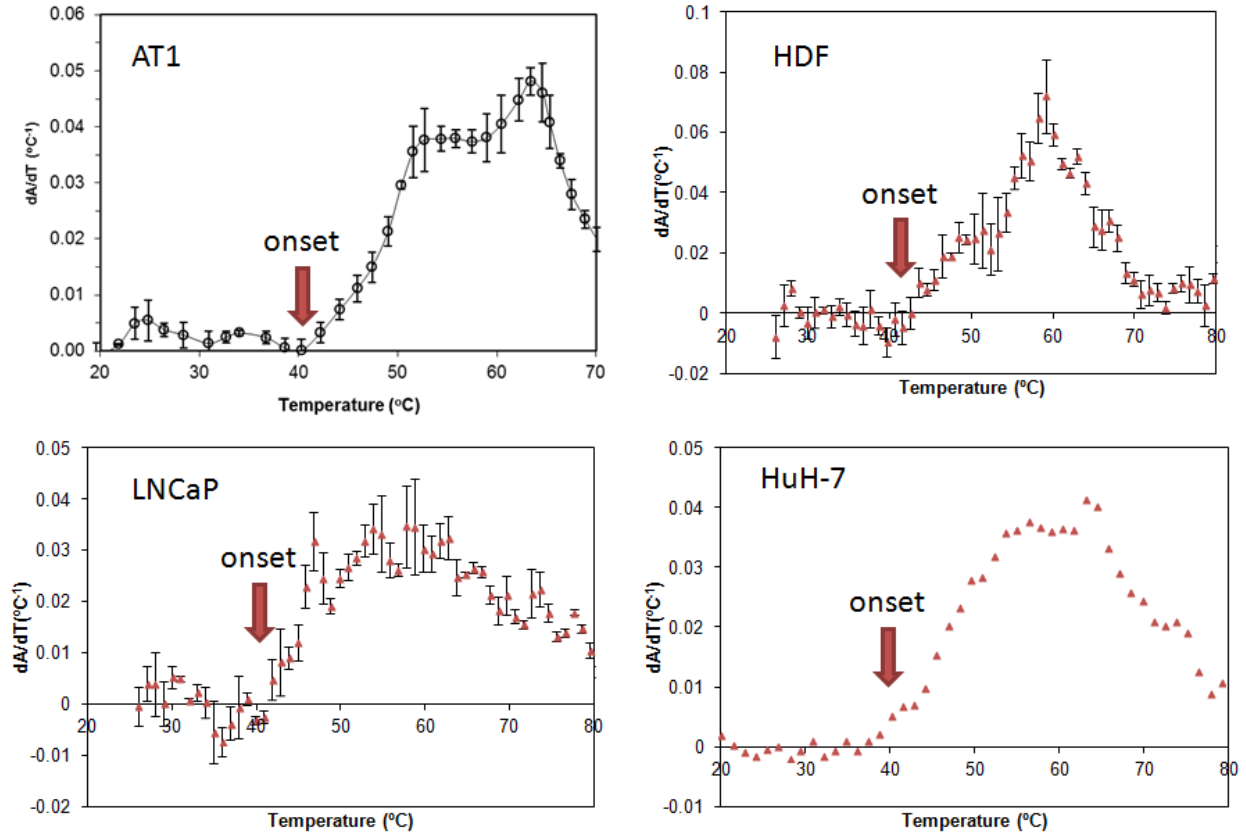
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## Supporting Information

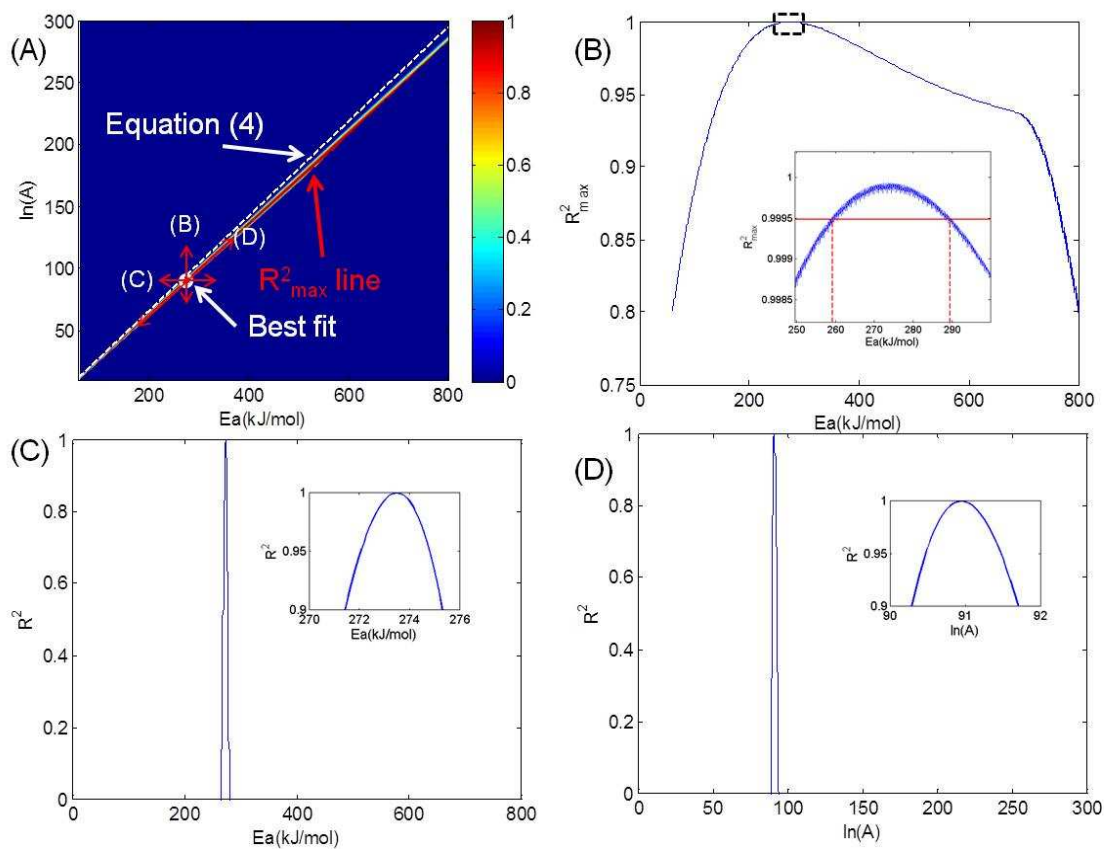
### S1. Determination of onset temperature for protein denaturation from FTIR (and DSC) measurements

The onset temperature was determined by taking the first derivative of the fractional denaturation and finding zero derivative at temperatures higher than 37°C. Examples on the cells are shown in Figure S1.



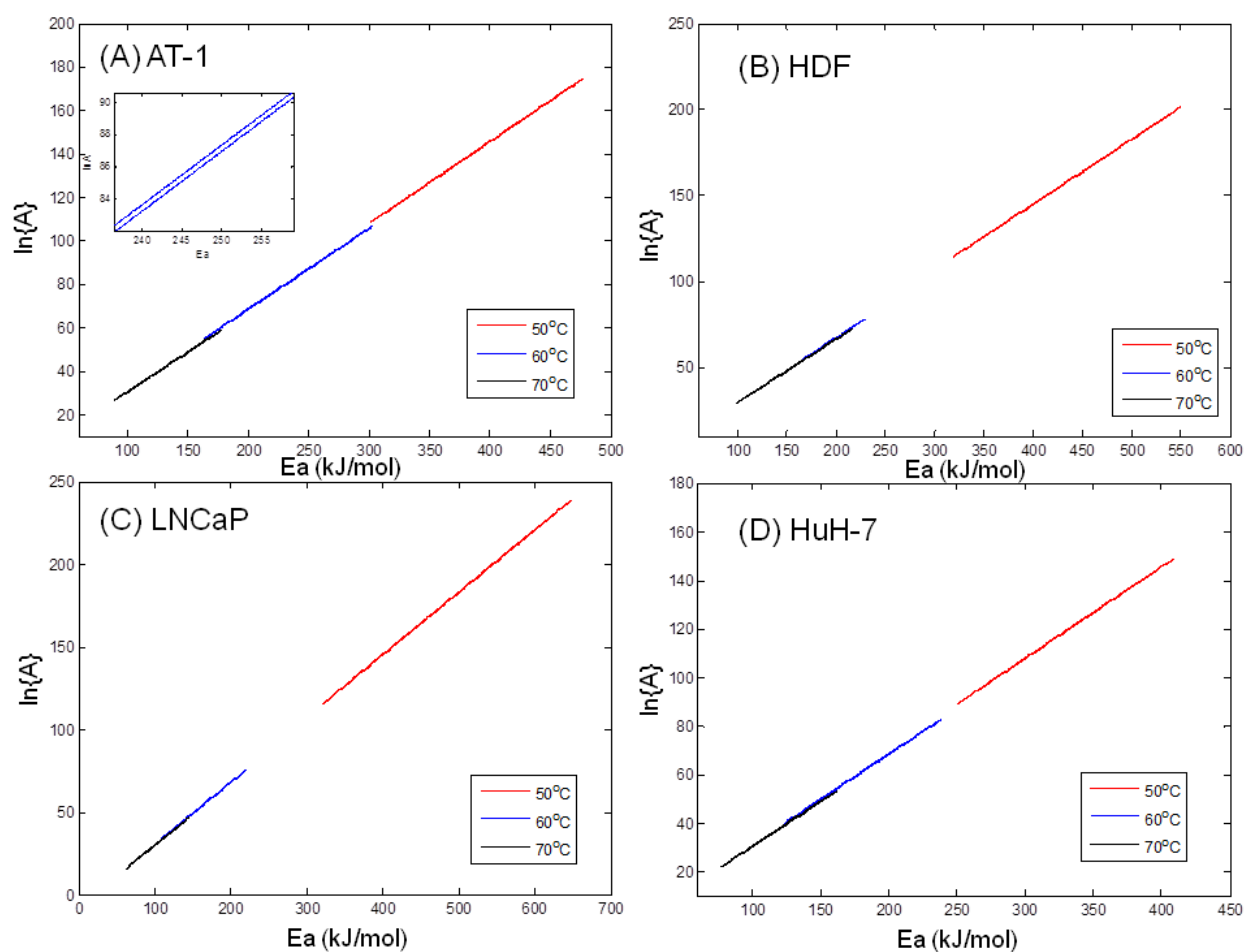
**Figure S1.** Determination of the onset temperature of protein denaturation for the four different cell lines investigated in this study.

## S2. Sensitivity analysis of the fitting



**Figure S2.** Analysis of the sensitivity and characteristics for the fitting. (A) shows the distribution of  $R^2$  for  $E_a$ - $\ln$  space for Miles et al.  $0.1^\circ\text{C}/\text{min}$ . There exists a high  $R^2$  region and a linear line can be plotted along the highest  $R^2$  ( $R^2_{\max}$  line). The  $R^2_{\max}$  line is close to Equation 4. The white circle corresponds to the best fit. (B) Distribution of the  $R^2_{\max}$  along the  $R^2_{\max}$  line. Inset shows the distribution of  $R^2_{\max}$  close to the optimal  $R^2_{\max}$  value. The distributions of  $R^2$  are also plotted with (C) the constant optimal  $\ln$  and (D) the constant optimal  $E_a$  value.

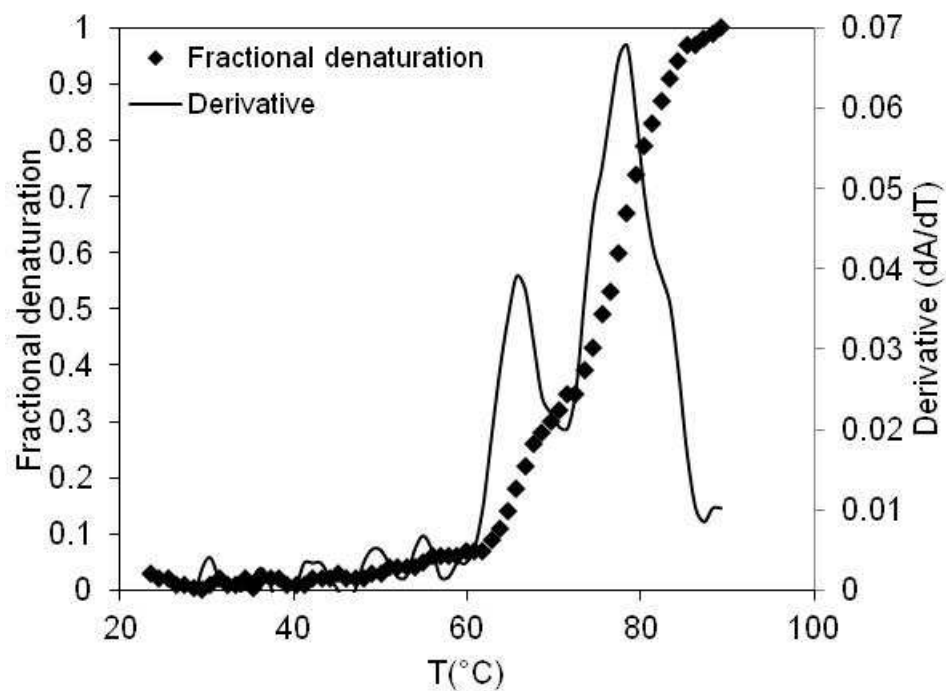
### S3. Confidence ellipse



**Figure S3.** Confidence ellipses for protein denaturation. Note that the ellipse has a high aspect ratio making it appear almost as a line.<sup>[1]</sup> The inset of (A) shows a closer look at the ellipse.

#### S4. Egg white denaturation

As shown in Figure S3, the denaturation of egg white (60-65% ovalbumin among other proteins<sup>[2]</sup>) displays two distinct peaks (peak at 65.7°C and 77.5°C respectively).



**Figure S4.** Thermal denaturation kinetics of egg white.

## S5. Protein Denaturation at High Temperatures

For protein denaturation at high temperatures ( $>90^{\circ}\text{C}$ ), we note that there are some deviations from the classical Arrhenius model. Specifically, calculation of  $\ln\{A\}$  from Equation (4) gives negative values, which disagrees with the MD simulation ( $\ln\{A\}= 26$  to  $36$ ) and is outside the tolerance range proposed in this study (*i.e.*  $C = -14.9$  to  $-16.4$ , Table 5). This is an area of active research and may have something to do with water phase change (*i.e.* boiling) in addition to the protein behavior.

**Table S1.** Arrhenius kinetic parameters from molecular dynamic simulations of high temperature protein denaturation ( $100 \sim 400^{\circ}\text{C}$ ).  $C$  is the parameter from Equation (8).

Protein	$E_a$ ( $\text{kJ mol}^{-1}$ )	$\ln\{A\}$	$C$	Ref.
EGF (TS1)	36.8	30.1	-16.1	[3]
EGF (TS2)	28.5	26.7	-15.8	[3]
EnHD	43.6	33.0	-16.4	[4]
$\alpha_3\text{D}$	33.1	27.9	-15.3	[4]
Cl2	56.7	36.5	-14.9	[4]

## References

1. Barrie, P. J., The mathematical origins of the kinetic compensation effect: 1. the effect of random experimental errors. *Phys. Chem. Chem. Phys.* 14: 318-326, 2012
2. Huntington, J. A. and Stein, P. E., Structure and Properties of Ovalbumin. *J. Chromatogr. B* 756: 189-198, 2001
3. Yan, C., Pattani, V., Tunnell, J. W. and Ren, P., Temperature-Induced Unfolding of Epidermal Growth Factor (EGF): Insight From Molecular Dynamics Simulation. *J. Mol. Graphics Modell.* 29: 2-12, 2010
4. Day, R. and Daggett, V., Sensitivity of the Folding/Unfolding Transition State Ensemble of Chymotrypsin Inhibitor 2 to Changes in Temperature and Solvent. *Protein Sci.* 14: 1242-1252, 2005