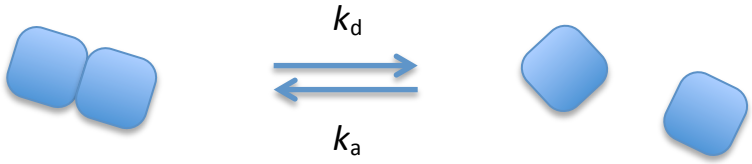
Activation Energy of Protein Dimerization

Origin_Assign_7_DimerDissoc_Data.xlsx contains the dissociation kinetics of a protein dimer (60 kDa), which dissociates slowly with time. In these experiments, the sample was prepared in a nonequilibrium initial state, consisting primarily of dimer, and the re-establishment of equilibrium was monitored as an increase in the fraction of monomeric protein over time.



$$P_2 \rightleftarrows 2P$$

$$K_d = \frac{k_d}{k_a} = \frac{[P]^2}{[P_2]} \tag{1}$$

In Equation (1), K_d is the equilibrium dissociation constant; k_d is the first order rate constant for dissociation and k_a is the second order rate constant for association. The dissociation kinetics have a very strong temperature dependence.

The Excel file contains the rate data for three different temperatures, 4, 14 and 30 ^{o}C. The kinetics of dimer dissociation can be fit to equation (2), which describes the fraction of protein in the dimeric state (f_d) as a function of time (t), the fraction of dimeric protein at the beginning of the experiment ($f_{d,0}$), the fraction of dimeric protein after equilibrium has been re-established ($f_{d,eq}$), and the rate constant for dimer dissociation (k_d)

$$f_d = \frac{f_{d,0} - f_{d,eq} + f_{d,eq}\left(1 - f_{d,0}f_{d,eq}\right)\exp\left(\frac{1+f_{d,eq}}{1-f_{d,eq}}k_d t\right)}{f_{d,eq}\left(f_{d,0} - f_{d,eq}\right) + \left(1 - f_{d,0}f_{d,eq}\right)\exp\left(\frac{1+f_{d,eq}}{1-f_{d,eq}}k_d t\right)} \tag{2}$$

$f_{d,0}$ = fraction of protein dimer at the beginning of the experiment (t = 0)
$f_{d,eq}$ = fraction of protein dimer upon re-establishing the equilibrium ($t \rightarrow \infty$)
k_d = first order rate constant for the dissociation of dimer

The fitting procedure provides direct estimates of $f_{d,0}$, $f_{d,eq}$ and k_d as adjustable parameters in the fit. From these values, estimates of K_d and k_a can be computed.

k_d, K_d and k_a were determined over a range of temperatures (Table 1), which allows transition state theory analysis of the rate data.

TABLE 1. Rate Data for the Dissociation of Protein Dimers[a]

no.	[P] (μM)[b]	T (°C)	$f_{d,eq}$[c]	K_d (μM)[c]	k_d x 10^6 (s^{-1})[c]	k_a ($M^{-1}s^{-1}$)[c]
1	2.9	4.0	0.53 ± 0.01	**	2.3 ± 0.2	**
2	4.2	9.0	0.48 ± 0.05	4.7 ± 0.5	3.6 ± 0.6	0.7 ± 0.1
3	4.0	14.0	0.43 ± 0.01	**	7.8 ± 0.7	**
4	1.9	18.0	0.34 ± 0.01	4.9	19 ± 2	4.0
5	3.4	23.0	0.32 ± 0.01	9.8	44 ± 3	4.3
6	7.9	23.0	0.46 ± 0.01	10	85 ± 5	8.3
7	3.1	27.0	0.26 ± 0.01	13	153 ± 10	12
8	3.1	30.0	0.22 ± 0.01	**	328 ± 17	**
9	6.0	30.0	0.28 ± 0.01	22	488 ± 17	22

[a]Monomer and dimer fractions *vs.* time were determined by gel-filtration, except experiments nos. 6 & 9, which were determined by circular dichroism spectroscopy.

[b]Protein concentrations are expressed in moles of monomer.

[c]Uncertainties in $f_{d,eq}$ and k_d are standard errors determined from curve-fitting. The uncertainties in k_a and K_d were determined by the propagation of error from the uncertainty estimates of $f_{d,eq}$ and k_d. Otherwise, uncertainties of 10% were assumed for k_a and K_d on the basis of the uncertainty in the total protein concentration.

In the transition state analysis of the data, Eyring plots are used to determine $\Delta H^{o\ddagger}$ and $\Delta S^{o\ddagger}$

$$\ln\left(\frac{k_d}{T}\right) = \ln\left(\frac{k_B}{h}\right) - \frac{\Delta G^{o\ddagger}}{RT} \tag{3}$$

If the plot of $\ln(k_d/T)$ vs. $1/T$ is not linear, then the expression for $\Delta G^{o\ddagger}$, which does not assume $\Delta C_P^{\ddagger} \approx 0$, should be used

$$\Delta G^{o\ddagger} = \Delta H^{o\ddagger} + \Delta C_P^{o\ddagger}\left(T - T_r\right) - T\left[\Delta S^{o\ddagger} + \Delta C_P^{o\ddagger}\ln\left(\frac{T}{T_r}\right)\right] \tag{4}$$

Procedure/Report Format

1. Use Origin to fit the 4, 14 and 30 °C kinetic data (in *Origin_Assign_7_DimerDissoc_Data.xlsx*) using equation (2). Your values of $f_{d,eq}$ and k_d should agree with (be similar to) those in Table 1.

2. From the estimates of $f_{d,eq}$ and k_d, compute K_d and k_a. (In other words fill in the ** to Table 1). Show the calculations. (Neat handwritten calculations, scanned, are fine to turn in.)

3. Create an Eyring plot ($\ln(k_d/T)$ vs. $1/T$) of the dissociation rate constants in Table 1 (also in the Excel file). Fit the data according to equations (3 & 4) to generate estimates $\Delta H^{o\ddagger}$, $\Delta S^{o\ddagger}$ and $\Delta C_P^{\ddagger}$ of dissociation. (Use a reference temperature of 298 K in this analysis.)

4. In a DSC study of protein stability, the monomeric protein was found to have calorimetric enthalpy and heat capacity changes (ΔH^o and ΔC_P) of 255 kJ/(mol monomer) and 4.2 kJ/deg/(mol monomer), respectively, at the T_M (60 °C). How do these values of compare to $\Delta H^{o\ddagger}$, $\Delta S^{o\ddagger}$ and $\Delta C_P^{\ddagger}$ at the same temperature. (Hint: use the $\Delta C_P^{\ddagger}$ to compute $\Delta H^{o\ddagger}$, $\Delta S^{o\ddagger}$ at the T_M.) What does this tell you about the character of the transition state for dissociation?

Answers

1. *Use Origin to fit the 4, 14 and 30 °C kinetic data (in Origin_Assign_7_DimerDissoc_Data.xlsx) using equation (2). Your values of $f_{d,eq}$ and k_d should agree with (be similar to) the values in Table 1.*

The fraction dimer data versus time at 4, 14 and 30 °C were fit to equation (2), which generated the fits that are shown in the Figure A1 and the values for $f_{d,eq}$ and k_d that are summarized in the Table A1.

Table A1. k_d and $f_{d,eq}$ estimated from data

°C	$f_{d,eq}$	k_d x 10^6 (s^{-1})
4	0.564 ± 0.013	2.503 ± 0.132
14	0.434 ± 0.010	7.889 ± 0.272
30	0.209 ± 0.005	209.26 ± 5.97

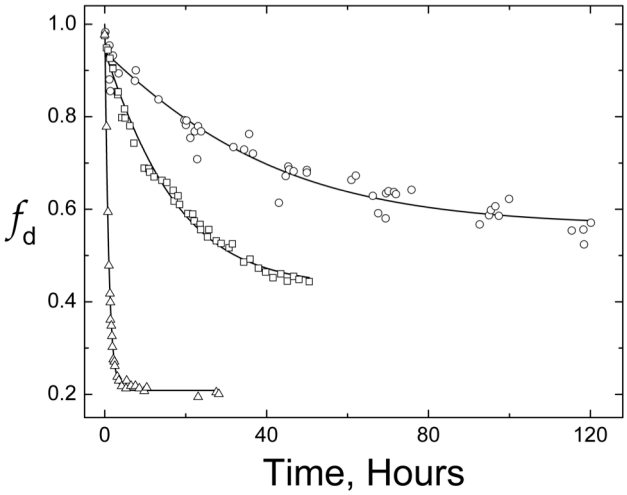


Figure A1. Fraction of dimer versus time at 4, 14 and 30 °C. (○, □ and △, respectively.)

2. *From the estimates of $f_{d,eq}$ and k_d, compute K_d and k_a. (In other words fill in the ** to Table 1). Show the calculations. (Neat handwritten calculations, scanned, are fine to turn in.)*

To determine K_d and k_a from $f_{d,eq}$ and k_d, the *total* protein concentration, $[P]_{tot}$, is used with conservation of mass equations to calculate K_d and then k_a (from $K_d = k_d/k_a$). The mass conservation equation between the fraction of monomer and fraction of dimer at equilibrium is

$$f_{m,eq} + f_{d,eq} = 1 \qquad \text{A1}$$

Eq. A1 is related to the mass conservation equation, Eq. A2, expressed with protein concentrations

$$[M] + 2[D_2] = [P]_{tot} \qquad \text{A2}$$

$[P]_{tot}$ is the total concentration of monomer, which is constant in any given experiment. The connection between Eqs. A1 & A2 becomes explicit after multiplying Eq. A1 through by $[P]_{tot}$.

$$f_{m,eq}[P]_{tot} + f_{d,eq}[P]_{tot} = [P]_{tot} \qquad \text{A3}$$

Comparing Eq. A2 and Eq. A3, the relationships between [M] and $[D_2]$, and $f_{d,eq}$ and $[P]_{tot}$, are observed

$$[M] = (1 - f_{d,eq})[P]_{tot} \qquad [D_2] = f_{d,eq}[P]_{tot}/2 \qquad \text{A4, A5}$$

The equation for the equilibrium constant, when written in terms of these relations, allows us to estimate K_d and k_a at 4, 14 & 30 °C from the values of $f_{d,eq}$, $[P]_{tot}$ and k_d at those temperatures.

$$K_d = \frac{k_d}{k_a} = \frac{[M]^2}{[D_2]} = \frac{2\left(1 - f_{d,eq}\right)^2 [P]_{tot}}{f_{d,eq}} \qquad \text{A6}$$

Example calculations with the 4 °C data are

$$K_d = \frac{2\left(1 - f_{d,eq}\right)^2 [P]_{tot}}{f_{d,eq}} = \frac{2\left(1 - 0.564\right)^2 2.9\ \mu M}{0.564} = 1.95\ \mu M$$

$$k_a = \frac{k_d}{K_d} = \frac{2.503\ x\ 10^{-6}\ s^{-1}}{1.95\ x\ 10^{-6}\ M^{-1}} = 1.29\ M^{-1}s^{-1}$$

Table A2 list the values of K_d and k_a at 4, 14 & 30 °C

TABLE A2. Equilibrium and kinetic parameters of protein dimerization

no.	[P] (μM)	T (°C)	$f_{d,eq}$	K_d (μM)	k_d x 10^6 (s^{-1})	k_a ($M^{-1}s^{-1}$)
1	2.9	4.0	0.564 ± 0.013	1.95	2.503 ± 0.132	1.29
3	4.0	14.0	0.434 ± 0.010	5.90	7.889 ± 0.272	1.34
8	3.1	30.0	0.209 ± 0.005	18.6	209.26 ± 5.97	11.2

3. *Create an Eyring plot, ln(k_d/T) vs. 1/T, of the dissociation rate constants in Table 1 (also in the Excel file). Fit the data according to equations (3 & 4) to generate estimates for $\Delta H^{o\ddagger}$, $\Delta S^{o\ddagger}$ and $\Delta C_P^{\ddagger}$ of dissociation. (Use a reference temperature of 298 K in this analysis.)*

In Eyring rate theory, k_d has a temperature dependence in both the pre-exponential factor and the exponent.

$$k_d = \frac{k_B T}{h} \exp\left(\frac{-\Delta G^{o\ddagger}}{RT}\right) \qquad \text{A7}$$

The pre-exponential term contains the expression for thermal energy, k_bT, scaled by the Planck constant, h, which has units of energy per frequency (*e.g.* J·$(s^{-1})^{-1}$). The Planck constant – frequency product is an energy, in which the frequency corresponds to the frequency at which the transition state forms and decomposes. This would be k_d, if it weren't for the exponential term ($-\Delta G^{o\ddagger}/RT$), which weights the rate according the energetic differences between reactant and transition states. The Eyring plot removes the temperature dependence from the pre-exponential term.

$$\ln\left(\frac{k_d}{T}\right) = \left[\ln\left(\frac{k_B}{h}\right) + \frac{\Delta S^{o\ddagger}}{RT}\right] - \frac{\Delta H^{o\ddagger}}{RT} \qquad \text{A8}$$

Substituting $\Delta H^{o\ddagger}$ - $T\Delta S^{o\ddagger}$ for $\Delta G^{o\ddagger}$ in Eq. 3 and rearranging gives Eq. A8. It can be seen that the Eyring plot is linear *provided that $\Delta H^{o\ddagger}$ and $\Delta S^{o\ddagger}$ are constant with temperature.* Before the advent of personal computers, the Eyring plot (as used in the 50s and 60s) made the analysis of activation enthalpy convenient, but today this is no longer necessary, and in this case the plot is not likely to be linear, as $\Delta C_P^{\ddagger}$ is expected to be nonzero.

$$\ln\left(\frac{k_d}{T}\right) = \ln\left(\frac{k_B}{h}\right) - \frac{\Delta H^{o\ddagger}}{RT} - \frac{\Delta C_P^{\ddagger}\left(T - T_r\right)}{RT} + \frac{T\left(\Delta S^{o\ddagger} + \Delta C_P^{\ddagger}\ln\left(T/T_r\right)\right)}{RT} \qquad \text{A9}$$

To fit the data in Origin, Eq. A9 should be cast in a fitting-routine-friendly format, *i.e.* identify the convenient forms for the variables and parameters, and group the terms according to the temperature dependence (independent of T, inversely proportional to T, etc.). These considerations lead to Eq. A10. The leading term in Eq. A10, $\ln(k_B/h)$, is a constant that can be evaluated with the numerical values of k_B and h. The two other temperature-independent terms, $\Delta C_P^{\ddagger}/R$ and $\Delta S^{o\ddagger}/R$, are grouped together with this. The 4th and 5th terms vary in proportion to inverse T, and the final term varies with the logarithm of inverse temperature (after a bit of algebraic manipulation). The dependent (y) variable is $\ln(k_d/T)$ and the independent variable (x) is $1/T$ (*invT*).

$$\ln\left(\frac{k_d}{T}\right) = \left(\ln\left(\frac{k_B}{h}\right) - \frac{\Delta C_P^{\ddagger}}{R} + \frac{\Delta S^{o\ddagger}}{R}\right) - invT*\left(\frac{\Delta H^{o\ddagger}}{R} - \frac{\Delta C_P^{\ddagger}}{R}*T_r\right) - \frac{\Delta C_P^{\ddagger}}{R}\ln\left(T_r*invT\right) \qquad \text{A10}$$

With the numerical values of k_B and h,

$h = 6.62607 \times 10^{-34}$ J·s $\quad (kg{\cdot}m^2/s^2){\cdot}s$ $\qquad\qquad$ $k_B = 1.38065 \times 10^{-23}$ J·K^{-1} $\quad (kg{\cdot}m^2/s^2){\cdot}K^{-1}$

the leading term takes on the value of -1.568 $(K{\cdot}s)^{-1}$

$$\ln\left(\frac{k_B}{h}\right) = \ln\left(\frac{1.38065\ x\ 10^{-23}\ \left(kg{\cdot}m^2/s^2\right){\cdot}K^{-1}}{6.62607\ x\ 10^{-23}\ \left(kg{\cdot}m^2/s^2\right){\cdot}s}\right) = -1.568457\ \left(K{\cdot}s\right)^{-1}$$

The adjustable parameters are all divided by the gas constant R. One approach, then, is to use these as the adjustable parameters, *i.e.* $\Delta C_P^{\ddagger}/R$, $\Delta S^{o\ddagger}/R$ and $\Delta H^{o\ddagger}/R$, written as *delCR*, *delSR* and *delHR*, respectively. The dependent and independent variables, and the fixed parameter are also given names that have an association to the variable.

$$\text{lnkbyT} = \left(-1.568457 - delCR + delSR\right) - \text{invT}*\left(delHR - delCR*Tref\right) - delCR*\ln\left(Tref*\text{invT}\right) \qquad \text{A11}$$

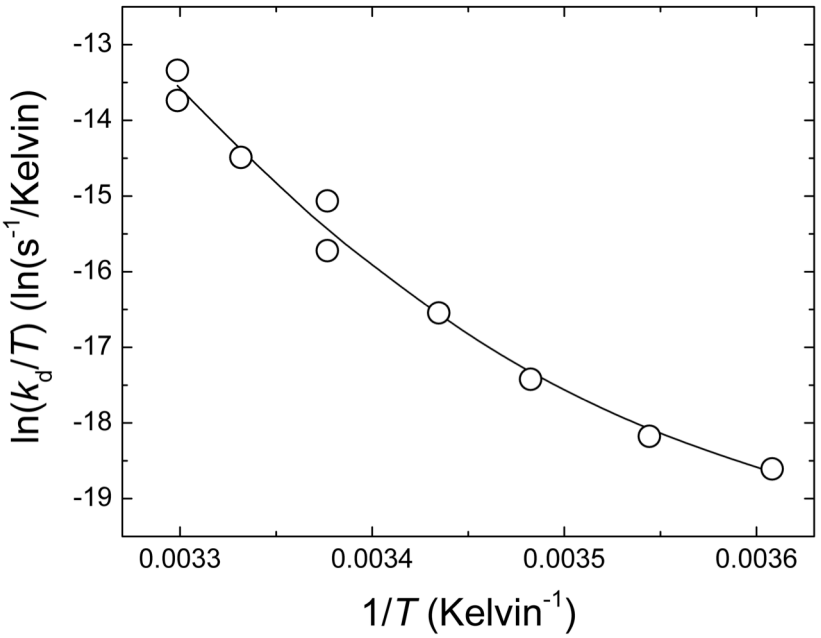


Figure A2. Eyring plot of dissociation rate data with fit according to Eq. A11.

The parameters returned by the fit in Origin (Table A3) are converted to the standard forms by multiplying through with *R* (Table A4). The values in Table A4 are expressed as per mole dimer (first row) and per mole monomer (second row).

TABLE A3. Fit Parameters for Dimer Dissociation at 298 *K*

delHR	***delSR***	***delCR***	***Tref***	**Red. χ^2**	**Adj. R^2**
23187.4 ± 1681.7 K	64.398 ± 5.636 K^2	809.72 ± 200.10 K^2	298 K	0.05359	0.98532

TABLE A4. Transition State Parameters for Dimer Dissociation at 298 *K*

	$\Delta H^{o\ddagger}$	$\Delta S^{o\ddagger}$	$\Delta C_P^{\ddagger}$	T_{ref}
per mole dimer	192.8 ± 14.0 kJ·mole^{-1}	535 ± 47 J·(mole·K)$^{-1}$	6.732 ± 1.664 kJ·(mole·K)$^{-1}$	298 K
per mole monomer	96.4 ± 7.0 kJ·mole^{-1}	268 ± 24 J·(mole·K)$^{-1}$	3.366 ± 0.832 kJ·(mole·K)$^{-1}$	298 K

4. *In a DSC study of protein stability, the monomeric protein was found to have calorimetric enthalpy and heat capacity changes (ΔH^o and ΔC_P) of 255 kJ/(mol monomer) and 4.2 kJ/deg/(mol monomer), respectively, at the T_M (60 ^{o}C). How do these values compare to $\Delta H^{o\ddagger}$, $\Delta S^{o\ddagger}$ and $\Delta C_P^{\ddagger}$ at the same temperature. (Hint: use the $\Delta C_P^{\ddagger}$ to compute $\Delta H^{o\ddagger}$, $\Delta S^{o\ddagger}$ at the T_M.) What does this tell you about the character of the transition state for dissociation?*

The changes in enthalpy, entropy and heat capacity when the protein unfolds at (61 ^{o}C) are:
ΔH^o = 255 kJ/mol
ΔS^o = 255 kJ/mol/334 K = 764 J/mol/K
ΔC_P = 4.2 kJ/mol/K

The changes in enthalpy, entropy and heat capacity when the protein dimer undergoes dissociation through the transition state were computed with a reference temperature of 298K; these are recalculated for 331K (61 ^{o}C) using the relationships that account for the temperature dependence of $\Delta H^{o\ddagger}$ and $\Delta S^{o\ddagger}$. Comparisons between dissociation and unfolding are made per mole of monomer.

Thermodynamic quantities for the formation of the transition state at 61 ^{o}C from dimer (per mole monomer):

$\Delta H^{o\ddagger}$(334 K) = $\Delta H^{o\ddagger}(298\ K) + \Delta C_P^{\ddagger}(T - T_{ref}) = 96.4$ kJ/mol + (3.36 kJ/mol/K)*(334-298) K = **217 kJ/mol**

$\Delta S^{o\ddagger}$(334 K) = $\Delta S^{o\ddagger}(298\ K) + \Delta C_P^{\ddagger}\ln(T/T_{ref}) = 268$ J/mol/K + (3366 J/mol/K)*ln(334/298) = **651 J/mol/K**

TABLE A5. Comparison of Thermodynamic Parameters for Protein Unfolding with formation of the Transition State from the Dimeric State (61 ^{o}C)

Protein Unfolding		**Dimer Dissociation**		**% (Dissoc./Unfold)**
ΔH^{o}	255 kJ/mol	$\Delta H^{o\ddagger}$	217 kJ/mol	85
ΔS^{o}	764 J/mol/K	$\Delta S^{o\ddagger}$	651 J/mol/K	85
ΔC_P	4.2 kJ/mol/K	$\Delta C_P^{\ddagger}$	3.36 kJ/mol/K	80

The comparison of thermodynamic properties provides evidence that the dimer dissociation process involves a transition state that is nearly unfolded, ~85%. This suggests that the dimeric state has extensive contacts – intersubunit contacts – that can only form if the protein goes through a transition state, which resembles unfolded protein. Although it is not typical of many situations involving protein oligomerization, it does have similarities to an important set of oligomerization reactions that involve 'domain swapping'. When domain swapping is involved, intramolecular domain-domain interactions in the monomer are broken and reform as intermolecular domain-domain interactions in the dimer.