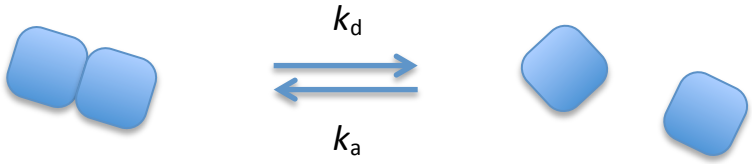
Activation Energy of Protein Dimerization

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 $\Delta C_P^{o\ddagger} \approx 0$, 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 $\Delta C_P^{o\ddagger}$ 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 $\Delta C_P^{o\ddagger}$ at the same temperature. (Hint: use the $\Delta C_P^{o\ddagger}$ 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?

EC1. Construct an Eyring plot with the values of k_a from Table 1 and obtain estimates of $\Delta H^{o\ddagger}$, $\Delta S^{o\ddagger}$ and $\Delta C_P^{o\ddagger}$ of association. Compute ΔH^o, ΔS^o and ΔC_P^o of dimer dissociation at the reference temperature (298 K). What do you learn of the differences between the monomeric and dimeric forms of the protein?

EC2. Derive equation (2). (All steps in the derivation must be shown; all terms in the derivation must be defined. Neat (legible) handwritten derivations are acceptable.)