1. Using Sterling's approximation ($\ln x! \approx x \ln x$), we can write the Gibb's free energy of creating n imperfections to a crystal of N atoms as:

$$G(n) = G(0) + nH_p - nT\Delta S_{th} - k_b T\{(N+n)\ln(N+n) - N\ln N - n\ln n\}$$

The minimum Gibb's free energy is found by equating the first differential to zero:

$$\frac{\partial G(n)}{\partial n} = H_p - T\Delta S_{th} - k_b T\{\ln(N+n) - \ln n\} = 0 \qquad \text{or} \qquad \ln\left(\frac{n}{n+N}\right) = -\frac{H_p}{k_b T} + \frac{\Delta S_{th}}{k_b}$$

For n ≪ N,

$$\frac{n}{N} = \exp\left(-\frac{H_p}{k_b T}\right)\exp\left(\frac{\Delta S_{th}}{k_b}\right).$$

2. We define conformation A to be the conformation in which the three residues are buried in the interior of the protein, and conformation B to be the one in which the three residues are exposed to the solvent. The free energy of transfer, ΔG_t, for Ala, Leu, and Phe are +0.73, +2.42, and +2.65 kcal/mole respectively. The sum of these free energy changes is $\Delta G_{AB} = +5.80$ kcal/mole and this is, to a first approximation, the free energy change associated with the transition from A to B in water. The equilibrium constant is given by

$$K_a = \frac{k_{A\to B}}{k_{B\to A}} = \frac{[B]}{[A]} = \exp\left(-\frac{\Delta G_{AB}}{RT}\right)$$

$$= \exp\left(-\frac{5.80 kcal/mol}{(0.001985 kcal/mol\cdot K)(298.15K)}\right)$$

$$= 5.57\times 10^{-5} \quad \text{or}$$

$$K_d = \frac{k_{B\to A}}{k_{A\to B}} = \frac{[A]}{[B]} = 1.794\times 10^4$$

As we are discussing a unimolecular reaction, the definition of association and dissociation constant is arbitrary. What is important is that the conformation [A] is more stable than conformation [B].

The free energies of transfer from water to Urea are -0.07, -0.38, and -0.70 kcal/mole for Ala, Leu, and Phe, respectively. Thus, when the transfers are made the ethanol like environment of the protein interior to urea, ΔG_{AB} is *reduced* by 1.15 kcal/mol with respect to water.

$$K_a = \exp\left(-\frac{(5.80-1.15)kcal/mol}{(0.001985kcal/mol{\cdot}K)(298.15K)}\right)$$

$$= 3.89 \times 10^{-4} \quad \text{or}$$

$$K_d = 2.57 \times 10^3$$

3. First, determine k_{on} assuming a diffusion limited rate constant:

$$k_{on} = 4\pi r_0(D_A + D_B)N_0 \times 10^{-3}s^{-1}M^{-1}$$
$$= 4\pi(30 \times 10^{-8})(90 \times 10^{-8})N_0 \times 10^{-3}s^{-1}M^{-1}$$
$$= 2.04 \times 10^9 s^{-1}M^{-1}$$

From the equilibrium constant, the off rate is given by:

$$k_{off} = k_{on}K_d = (2.04 \times 10^9 \text{ s}^{-1}\text{M}^{-1})(5 \times 10^{-15}\text{M}) = 1.02 \times 10^{-5} \text{ s}^{-1}$$

or $\quad \frac{1}{k_{off}} = 27.2$ hrs.

For a dissociation constant $K_d = 5$ nM and assuming a diffusion limited on rate,

$$k_{off} = k_{on}K_d = (2.04 \times 10^9 s^{-1}M^{-1})(5 \times 10^{-9}M) = 10.2s^{-1} \text{ or } \frac{1}{k_{off}} = 0.098 \text{ s}$$

4. Starting from transition state theory, reaction kinetics is described in terms of an activated complex, which is populated with a probability

$$\exp\left(-\frac{\Delta G^{\ddagger}}{RT}\right)$$

and an attempt frequency ν. In general,

$$k = \frac{1}{\tau} = \nu \exp\left(-\frac{\Delta G^{\ddagger}}{RT}\right)$$

To relate this expression to the single molecule folding measurements, the probability of being

in the activated state is given by $\quad \exp\left(-\frac{\Delta}{k_B T}\right)$

where $\Delta = \Delta G^{\ddagger}/N_0$ (and N_0 is Avogadro's number). The vibrational frequency at the bottom of the unfolded state is proportional to the reconfiguration time of the unfolded polymer, τ_0. We approximate ν_{min} as: $\nu_{min} \approx 1/(2\pi\tau_0)$. The attempt frequency of the *activated* complex is given by

$$\nu_{max} = \frac{\omega_{max}}{\omega_{min}}\nu_{min},$$

where $\omega_{max}/\omega_{min}$ is the ratio of the vibration frequencies in the activated state to that in the bottom of the unfolded state. Hence,

$$k = \frac{\omega_{max}}{2\pi\omega_{min}\tau_0}\exp\left(-\frac{\Delta}{k_B T}\right) = \frac{1}{\tau_f}$$

5. For the given reaction, the rate law gives:

$$V = k_2[X_1] + k_2'[X_2]$$

Under steady state conditions,

$$\frac{[E][S]}{[X_1]} = K_1 \quad \text{and} \quad \frac{[E][S]}{[X_2]} = K_1'.$$

The can be rearranged as:

$$[E] = K_1\frac{[X_1]}{[S]} = K_1'\frac{[X_2]}{[S]}$$

$$\text{Thus, } [X_2] = \frac{K_1}{K'}[X_1]$$

Writing $[E]$ in terms of the total enzyme concentration minus what is bound to substrate in the intermediate states, we have:

$$[E]_0 - [X_1] - [X_2] = K_1\frac{[X_1]}{[S]}$$

$$[E]_0 - [X_1] - \frac{K_1}{K'}[X_1] = K_1\frac{[X_1]}{[S]}$$

Solving for $[X_1]$ yields:

$$[E]_0 = [X_1]\left(1 + \frac{K_1}{K'} + \frac{K_1}{[S]}\right)$$

$$[X_1] = \frac{[E]_0}{\left(1 + \frac{K_1}{K'} + \frac{K_1}{[S]}\right)}$$

Similary, one can solve for $\left[X_2\right]$.

Substituting for $[X_1]$ and $[X_2]$ in the velocity relationship, we have:

$$V = \frac{k_2[E]_0}{\left(1 + K_1/[S] + K_1/K_1'\right)} + \frac{k_2'[E]_0}{\left(1 + K_1'/[S] + K_1'/K_1\right)}$$

We wish to see if we can rewrite this equation is terms of the solution for the second chemcial equation, where the reaction law yields:

$$V = \frac{V_S}{\left(1 + K_S/[S]_0\right)} \quad .$$

To see if it is possible to reform the equation, we first solve for V_{max} by letting $[S]$ go to ∞.

$$V\big|_{[S]\to\infty} = V_{max} = \frac{k_2[E]_0}{\left(1 + K_1/K_1'\right)} + \frac{k_2'[E]_0}{\left(1 + K_1'/K_1\right)}$$

$$= \frac{\left(K_1'k_2 + K_1 k_2'\right)[E]_0}{\left(K_1 + K_1'\right)}$$

Using the above expression for V_{max}, we can rewrite the equation for V as:

$$V = \frac{V_{max}}{\left(1 + \dfrac{K_1 K_1'}{K_1 + K_1'}\Big/[S]_0\right)} \quad .$$

Hence, it is not possible from the kinetics to distinguish the two types of reactions.

6. $\dfrac{dI}{I} = \dfrac{-P\cdot\pi\cdot r^2\cdot c\cdot N_A}{1000}\cdot dl = \dfrac{-\sigma\cdot c\cdot N_A}{1000}\cdot dl$

   Integration over a finite path-length l gives: $\ln\left(\dfrac{I}{I_0}\right) = \dfrac{-\sigma\cdot c\cdot N_A}{1000}\cdot l$

   Using Lambert-Beer's-Law we get: $A = -\lg\left(\dfrac{I}{I_0}\right) = \dfrac{-\ln\left(\frac{I}{I_0}\right)}{2.303} = \varepsilon\, c\, l$

   Hence: $\dfrac{\sigma\cdot c\cdot N_A}{1000\cdot 2.303}\cdot l = \varepsilon\, c\, l$

   $\Rightarrow \quad \varepsilon = \dfrac{\sigma\cdot N_A}{2303}$

   b) Extinction coefficient: $\varepsilon = \dfrac{6.537\cdot 10^{-14} cm^2\cdot 6.022\cdot 10^{23}\frac{1}{mol}}{2303} = 17093\,\dfrac{1}{M\ cm}$

   Absorbance of a 7 μM solution: $A = \varepsilon\, c\, l = 17093\,\dfrac{1}{M\ cm}\cdot 7\cdot 10^{-6}\ M\cdot 1\ cm = 0.120$