Midterm Take-Home Exam

GOOD LUCK!

<table>
<thead>
<tr>
<th>PROBLEM</th>
<th>POSSIBLE POINTS</th>
<th>SCORE</th>
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<td>TOTAL</td>
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(i) $\text{NAD}^+ + 2\text{H}^+ + 2\text{e}^- \rightarrow \text{NADH} + \text{H}^+$
Reverse: $\text{NADH} + \text{H}^+ \rightarrow \text{NAD}^+ + 2\text{H}^+ + 2\text{e}^-$

$\varepsilon'_{1} = -0.190\,V + \frac{0.059}{2} \log \left( \frac{\text{NAD}}{\text{NAD}^+} \right)$
$\varepsilon'_{2} = -0.320\,V + \frac{0.059}{2} \log \left( \frac{\text{NADH}}{\text{NAD}^+} \right)$

$\varepsilon'_{1} = -0.255\,V \quad \varepsilon'_{2} = -0.255\,V$

Add two $1/2$-Rxn $\Rightarrow \Delta \varepsilon' = 0\,V$.
So $\Delta G = 0$ at equilibrium already!

(ii) $\varepsilon'_{1} = -0.190\,V + \frac{0.059}{2} \log \left( \frac{1}{1000} \right) = -0.279\,V$
$\varepsilon'_{2} = -0.320\,V + \frac{0.059}{2} \log \left( 1000 \right) = -0.231\,V$

$\varepsilon'_{2} > \varepsilon'_{1}$ so $\Delta \varepsilon' = -0.231 - (-0.279) + 0.048\,V$

Rxn goes: $\text{NAD}^+ + \text{Lac} \rightarrow \text{NADH} + \text{PyR} + \text{H}^+$
$\Delta G = -n F \Delta \varepsilon' = -(2)(23.06\text{ kcal/mole})(0.048\,V)$

$\Delta G = -2.21\,\text{kcal/mole}$

$\Delta G = 9.26\,\text{joules/mole}$

$\varepsilon'_{1}: 0.048\,V$
$\varepsilon'_{2}: 0.0\,V$ (equilibrium)
2. \[ E + S \xrightleftharpoons{K_s}{\kappa} ES \xrightarrow{k_{p}} E + P + I \]

\[ EI + S \xrightarrow{\alpha K_s} EIS \xrightarrow{\kappa_P} E + I + P \]

First consider: \( \alpha = \beta; \beta < 1 \)

For \( I > 0 \), then new slope: \( \frac{1}{V_m \cdot \beta} = \frac{1}{V_m} \cdot \alpha = \frac{1}{V_m} \cdot \frac{K_m}{V_m} = \frac{K_m}{V_m} \) (no change) \( \Rightarrow \) parallel line, as \( \alpha = \beta \).

For \( \frac{K_m}{V_m} \beta \), where \( \beta < \alpha \), then the slope increases for \( I > 0 \) and the line pivots:

\[ \frac{1}{V_m} \rightarrow \frac{k_{p}}{V_m} \]

"partial mixed-type inhibiting"
3. A few:

- Ionic charge (electrostatic) stabilization (enthalpy)
- Proximity/orientation (entropy)
- Nucleophilic catalysis
- General acid/base catalysis
- "Strain" catalysis
- Stereospecificity +
  - Stereoisomerism
  - Stereochemistry
- A local pH and/or pKa's
- A local electrochemical potential
- Desolvation (vol)

2. Any examples that fit are acceptable
any, to the catalysis. Could the role of serine be tested
stereochemically? Is PIX a relevant phenomenon? Why or why not?

Pi + E ⇌ 3.6 mg

1. Facilitate for activation? [pH opt: 8]

2. Zn₂⁺ acts as a Lewis acid to bind O⁻ species
   and to stabilize + which has increasing O⁻ fields.

3. Phosphoenzyme intermediate. K₃ in slow step,
   (at low pH). E only forms dimers!

4. Ser provides active E nucleophile, -OH.

5. E-O⁻⁺ [E⁻⁺OH⁻] → E⁻ raising intermediate

7. \( \text{NH}_2\text{OH} \) intercepts common intermediate (phospho-E) competing with \( \text{H}_2\text{O} \); this reaction is faster (slightly) than hydrolysis.

\[
\text{E} \rightarrow \text{E} + \text{Pi} \\
\text{E} \rightarrow \text{E} + \text{NH}_2\text{OH} \text{ (Hydrolysis)}
\]

\[k_{\text{obs}} = k_3 + k_7\] (additive)

8. Hydrolysis step is rate determining at lower pH; faster at alkaline pH.

9. Enzyme cannot cleave C-P bond.
   (not a "phosphomimetic")

\[\text{Zn}^2+: (i) 2 \rightarrow \text{Zn}^2+ (ii) \text{enzyme ligands to Zn}.
\]

\[\text{Ser: general base to activate (as in mech.)}\]

\[\text{Test: use S- or } \text{in serine} \leftrightarrow \text{in S-}
\]

\[\text{direct rx. } \text{H}_2\text{O} \rightarrow \text{Si} - \text{S}
\]

10. Pick: \( \text{NO} \rightarrow \text{need extra}\)

\[\text{H}_2\text{O} \leftrightarrow \text{O} \leftrightarrow \text{O} \leftrightarrow \text{H} \leftrightarrow \text{X} \]

\[\text{NO} \leftrightarrow \text{O} \leftrightarrow \text{O} \leftrightarrow \text{H} \leftrightarrow \text{X} \]

\[\text{(in reformed substrate)}\]
5. The enzyme lactate dehydrogenase catalyzes the reaction

\[
\text{CH}_3\text{C}=\text{COO}^- + \text{NADH} + \text{H}^+ \rightarrow \text{CH}_3\text{C}=\text{COO}^- + \text{NAD}^+ \quad \text{Pyruvate} \quad \text{Lactate}
\]

NADH and NAD\(^+\) are the reduced and oxidized forms, respectively, of the coenzyme NAD. Solutions of NADH, but not NAD\(^+\), absorb light at 340 nm. This property is used to determine the concentration of NADH in solution by measuring spectrophotometrically the amount of light absorbed at 340 nm by the solution. Explain how these properties of NADH can be used to design a quantitative assay for lactate dehydrogenase.

\[
-\frac{d\text{NADH}}{dt} = -\frac{d\text{Pyruvate}}{dt} = \frac{d\text{NAD}^+}{dt} = \frac{d\text{Lactate}}{dt} = k_2 [\text{NADH}] [\text{Pyruvate}]
\]

For enzyme assays we measure initial rates when conditions (concentrations) are set to give a pseudo-linear change in [NAD\(^+\)].

Then rate \( \frac{d[\text{NADH}]}{dt} = \frac{\Delta A}{\Delta t} \) from Beer's law, \( \varepsilon = \frac{A}{\varepsilon \cdot l} \); monitor reaction at 340 nm (in NAD\(^+\) or protein or pyruvate/lactate interference).
6a. \[ \frac{S}{V} = \frac{K_m}{V_m} + \frac{S}{V_m} \]

\( V: \text{mM/min} \)

\[ \text{Plot data, get: } K_m = 0.598 \text{ mM} \]
\[ V_m = 51.5 \text{ mM.min}^{-1} \]

6b. \[ V = \frac{K_m \cdot V}{S} + V_m \]

\[ \text{E-H} \]

Note: CI \( \rightarrow \Delta V_m, \text{not } \Delta K_m, \text{ a slope only.} \)

NCI \( \rightarrow \Delta K_m, \text{not } \Delta V_m, \text{ a y intercept only.} \)

\[ \text{Plot data, get: } V_m' = 55.2 \text{ mM.min}^{-1} \sim 52 \text{ (Vm)} \]
\[ K_m' = 0.93 \pm K_m \]

\[ \therefore CI \]
7. The following experimental data were collected during a study of the catalytic activity of an intestinal peptidase with the substrate glycylglycine:

\[
\text{Glycylglycine} + \text{H}_2\text{O} \rightarrow 2 \text{glycine}
\]

<table>
<thead>
<tr>
<th>[S] (mM)</th>
<th>Product formed (μmol/min)</th>
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</thead>
<tbody>
<tr>
<td>1.5</td>
<td>0.21</td>
</tr>
<tr>
<td>2.0</td>
<td>0.24</td>
</tr>
<tr>
<td>3.0</td>
<td>0.28</td>
</tr>
<tr>
<td>4.0</td>
<td>0.33</td>
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<tr>
<td>8.0</td>
<td>0.40</td>
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<tr>
<td>16.0</td>
<td>0.45</td>
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Use graphical analysis (L-B) to determine the \( V_{\text{max}} \) and \( K_m \) for this enzyme preparation and substrate.

\[
\text{Plot:} \quad 1/V \\
\frac{1}{V_{\text{max}}} = \frac{1}{V_{\text{max}}} \quad \frac{1}{[S]} = \frac{1}{K_m} \quad \frac{1}{V_{\text{max}}} = \frac{1}{[S]} + \frac{1}{K_m} \\
V_{\text{max}} = 0.51 \mu\text{mol}\cdot\text{min}^{-1} \\
(\text{gly}) = 0.26 \text{mM} \quad (\text{gly}, \text{gly}) \\
\text{Thus,} \quad K_m = 2.2 \text{mM}
\]

\[
\begin{align*}
E + S & \xrightleftharpoons{h_1} ES \xrightarrow{k_-} E + P \\
& \text{ES} \xrightarrow{k_i} E + I \\
& \text{ESI}
\end{align*}
\]

\[
\begin{align*}
ev &= E + ES + ESI \\
k_i &= \frac{ES\cdot I}{ESI} ; \quad ESI = \frac{ES\cdot I}{k_i}
\end{align*}
\]

So \(\nu = E + ES + ES\cdot I/k_i\)

\[
e = e_0 - ES\left(1 + I/k_i\right)
\]

\[
dES/dt = -k_1 ES - k_2 ES
\]

\[
v = k_2 \cdot ES
\]

\[
E_0 \cdot E\cdot S = a(k_1 + k_2) \cdot ES
\]

\[
h_1 (e_0 - ES\cdot F) \cdot S = 0
\]

\[
h_1 \cdot e_0 \cdot S - h_1 ES\cdot F\cdot S = 0
\]

\[
(h_1 + k_2) ES + h_1 ES\cdot F\cdot S = \frac{h_1 e_0 \cdot S}{k_1}
\]

\[
ES \left[\frac{(h_1 + k_2) + F\cdot S}{k_1}\right] = e_0 \cdot S
\]

\[
\therefore \quad v = \frac{k_2 e_0 \cdot S}{\left[km + F\cdot S\right]}
\]

\[
LB: \quad \frac{1}{v} = \frac{km}{V_m} \left(\frac{I}{V_m}\right) + \frac{(I+I/k_i)}{V_m}
\]

\[
\frac{km}{V_m} \left(\frac{I}{V_m}\right) - \frac{(I+I/k_i)}{V_m} = \frac{i}{V_m}
\]
9. Explain supersecondary structure and give 3 examples of common protein motifs.

- (α-helix) (interchangeable)

- Particularly stable arrangements of several elements of secondary structure, and their connections.

- Polypeptides ≥ 1-200 residues, often folded into ≥ 2 stable globular units ("domains"). In smaller proteins, domain is the protein.

S.G. 1)

[β-α-β loop + α-α corner to exclude Heo from R = hydrophobic; separate α, β regions]
4) β barrel
5) twisted β sheet
6) α/β barrel
Protein Motifs Are the Basis for Protein Structural Classification

As we have seen, the complexities of tertiary structure are decreased by considering substructures. Taking this idea further, researchers have organized the complete contents of databases according to hierarchical levels of structure. The Structural Classification of Proteins (SCOP) database offers a good example of this very important trend in biochemistry. At the highest level of classification, the SCOP database borrows a scheme already in common use, in which protein structures are divided into four classes: all α, all β, α/β (in which the α and β segments are interspersed or alternate), and α + β (in which the α and β regions are somewhat segregated) (Fig. 6–22).

**figure 6–20**
Stable folding patterns in proteins. (a) Two simple and common motifs that provide two layers of secondary structure. Amino acid side chains at the interface between elements of secondary structure are shielded from water. Note that the β strands in the β-α-β loop tend to twist in a right-handed fashion. (b) Connections between β strands in layered β sheets. The strands are shown from one end, with no twisting included in the schematic. Thick connections are those at the ends nearest the viewer; thin connections are at the far ends of the β strands. The connections on a given end (e.g., near the viewer) do not cross each other. (c) Because of the twist in β strands, connections between strands are generally right-handed. Left-handed connections must traverse sharper angles and are harder to form. (d) Two arrangements of β strands stabilized by the tendency of the strands to twist. This β barrel is a single domain of α-hemolysin from the bacterium Staphylococcus aureus. The twisted β sheet is from a domain of photolyase from E. coli.

**figure 6–21**
Constructing large motifs from smaller ones. The α/β barrel is a common motif constructed from repetitions of the simpler β-α-β loop motif. This α/β barrel is a domain of the enzyme pyruvate kinase from rabbit.