You may use your notes and texts for this exam. There are three questions, and the total number of points is 150. Explaining your reasoning in detail and showing intermediate steps in calculations will maximize the partial credit awarded for answers that are not completely correct.
1. (50 points) Consider a model affinity selection experiment. A protein of interest is covalently attached to a solid support, and seven different ligands (in large excess over the molar amount of protein) are present in solution. The ligands bind reversibly to the protein. Their association constants and concentrations are:

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Association Constant (M$^{-1}$)</th>
<th>Concentration (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1000</td>
<td>1x10$^{-3}$</td>
</tr>
<tr>
<td>B</td>
<td>5000</td>
<td>2x10$^{-4}$</td>
</tr>
<tr>
<td>C</td>
<td>2.5x10$^4$</td>
<td>4x10$^{-5}$</td>
</tr>
<tr>
<td>D</td>
<td>1.25x10$^5$</td>
<td>8x10$^{-6}$</td>
</tr>
<tr>
<td>E</td>
<td>6.25x10$^5$</td>
<td>1.6x10$^{-6}$</td>
</tr>
<tr>
<td>F</td>
<td>3.1x10$^6$</td>
<td>3.2x10$^{-7}$</td>
</tr>
<tr>
<td>G</td>
<td>3.1x10$^7$</td>
<td>3.1x10$^{-8}$</td>
</tr>
</tbody>
</table>

After the immobilized protein is equilibrated in the ligand mixture, the free ligands are washed off and the bound ligands are analyzed.

A. (20 points) What fraction of the immobilized protein is bound to ligands with dissociation constants <1 $\mu$M? Defining the enrichment as the ratio between the fraction of species in the original mixture and the fraction bound to the protein, what is the enrichment of these ligands?

B. (15 points) Suppose that the total amount of protein immobilized is 1 pmol, and that the bound ligands are eluted into a volume of 100 $\mu$L and equilibrated again with immobilized protein (this time, 0.01 pmol, in order to ensure that ligand is in large molar excess over protein). What fraction of the immobilized protein is bound to ligands with dissociation constants <1 $\mu$M after this second round?

C. (15 points) Assume that you are able to detect ~10 femtomoles of ligand. How many rounds of selection will be possible using this technique as described? How might it be possible to extend the number of rounds if the ligands are small (~100 bases) nucleic acid aptamers?
2. (50 points) Many enzymatic reactions are reversible. To understand self-splicing by ribozymes, Herschlag and co-workers have examined ribozyme-catalyzed RNA cleavage in both the forward and the reverse direction. In the forward direction, guanosine or a nucleotide with guanosine at the 3’ end acts as a nucleophile, cleaving the 3’ A from a substrate nucleotide and adding it to the 3’ end of the guanosine-containing nucleotide.

Consider the following reaction where UCG is the guanosine nucleophile, the oligonucleotide CCCUCUA is the other substrate (“S”), and E is the ribozyme:

\[
\begin{align*}
\text{E} & \underset{k_1}{\overset{k_{+1}}{\rightleftharpoons}} \text{E}_{\text{UCG}} & \text{E}_{\text{UCG}} & \underset{k_2}{\overset{k_{+2}}{\rightleftharpoons}} \text{E}_{\text{UCGA}} & \text{E}_{\text{UCGA}} & \underset{k_3}{\overset{k_{+3}}{\rightleftharpoons}} \text{E} + \text{P}
\end{align*}
\]

Herschlag and co-workers equilibrated E with radiolabeled S. They then initiated the ribozyme reaction by addition of UCG (to form the E-S-UCG complex) and a large excess of unlabeled P (to prevent rebinding of radiolabeled S). They monitored the formation of radiolabeled product.

A. (25 points) Write expressions for the observed rate constant for product formation and the fraction of initially formed E-S-UCG complex that partitions to product. (Assume that the binding of UCG to the ribozyme is rapid enough that it does not contribute to the observed kinetics and that the amount of E-P-UCGA complex that partitions back to the E-S-UCG complex is negligible).

B. (10 pts) If \( k_{\text{obs}} = 5 \text{ s}^{-1} \) and the fraction of ES complex that partitions to product is 0.8, what are the values of \( k_2 \) (the rate constant for the chemical step in the forward direction) and \( k_1 \) (the rate constant for dissociation of S)?

C. (5 pts.) Experiments using radiolabeled P provide a value for \( k_3 \) (the rate constant for the chemical step in the reverse direction) of 1 \( \text{s}^{-1} \). What is the equilibrium constant for product formation at the ribozyme active site?

D. (10 pts.) The equilibrium constant for formation of P and UCGA from S and UCG in the absence of ribozyme is 2. What might account for the difference between the equilibrium constant on the ribozyme and the equilibrium constant for the unbound reagents?
3. Consider an ion channel that can be activated (opened) by binding of a neurotransmitter ligand, but which can also undergo desensitization, a process in which the ligand-bound open channel enters a long-lived non-conducting state. In the presence of saturating concentrations of the ligand (L), the conformational changes can be described by two coupled equilibria:

\[
\begin{align*}
\text{CL} & \xrightarrow{\alpha} \text{OL} & \xrightarrow{\beta} \text{DL} \\
\text{OL} & \xrightarrow{\gamma} \text{DL} & \xrightarrow{\delta} \text{CL}
\end{align*}
\]

where CL is the closed (non-conducting) state, OL is the open (conducting) state, and DL is the long-lived desensitized (also non-conducting) state.

A. (15 pts.) In the presence of saturating concentrations of ligand, the channel exhibits bursts of openings and closings that alternate with long sojourns in the desensitized state:

You observe a single mean open lifetime of 9.1 ms. The probability density function of the lifetimes of non-conducting states has two components: one with a mean lifetime of 0.5 ms and an amplitude of 0.91 (i.e., it makes up 91% of closings), and one with a mean lifetime of 1 s and an amplitude of 0.09. From these values, find values for \(\alpha\), \(\beta\), \(\gamma\), and \(\delta\).

B. (5 pts.) You discover that patients with an autoimmune disorder develop antibodies to this ion channel. In the presence of these auto-antibodies, the channel exhibits different kinetic properties:

You observe a single mean open lifetime of 9.1 ms. The probability density function of the lifetimes of non-conducting states has two components: one with a mean lifetime of 0.5 ms and an amplitude of 0.91 (i.e., it makes up 91% of closings), and one with a mean lifetime of 1 s and an amplitude of 0.09. From these values, find values for \(\alpha\), \(\beta\), \(\gamma\), and \(\delta\).
Question 3, continued.

In the presence of the antibody, the open lifetime is 91 ms. The probability density function of the lifetimes of non-conducting states in the presence of antibody exhibits one component with a mean lifetime of 0.5 ms and an amplitude of 0.91 and another component with a mean lifetime of 1 s and an amplitude of 0.09. Assume that in the presence of the antibody, the channel is completely bound to antibody. Determine values for $\alpha$, $\beta$, $\gamma$, and $\delta$ under these conditions.

C. (25 pts.) Assume that the antibody binds to one and only one conformation of the channel. Are these data most consistent with the antibody binding to the closed state, the open state, the desensitized state? How do the data rule out the hypotheses you do not favor? Explain.

Antibody binding may perturb the energetics of ground states of the channel only, or it may perturb the energetics of both ground states and transition states. Which possibility do these data favor? Explain.

D. (5 pts.) Based on your answer for which conformation of the channel binds the antibody (closed, open, or desensitized), how would you expect severity of attacks of the autoimmune disease to correlate with release of neurotransmitter? Explain.
You may use your notes and texts for this exam. The total number of points is 50. Explaining your reasoning in detail and showing intermediate steps in calculations will maximize the partial credit awarded for answers that are not completely correct.
1. (50 points) Consider a motor enzyme that obeys Michaelis-Menten kinetics:

\[
    v = \frac{k_{\text{cat}}(F)[S]}{[S] + \left[\frac{k_{\text{cat}}(F)}{k_b(F)}\right]}
\]

where \(v\) is the rate at which the motor takes a step, \(k_{\text{cat}}(F)\) is the turnover number (which is a function of applied force), \([S]\) is the substrate concentration, and \(k_b(F)\) is the apparent second order rate constant (equal to \(k_{\text{cat}}/K_m\), and also a function of applied force).

A. (10 pts.) Assume that both \(k_{\text{cat}}\) and \(k_b\) represent only mechanical steps in the catalytic cycle (as would be the case if chemical steps are fast compared to mechanical steps). Assume also that \(k_{\text{cat}}\) and \(k_b\) depend exponentially on force in a way analogous to the bond rupture rate constants discussed in class, but that, unlike those rate constants, \(k_{\text{cat}}\) and \(k_b\) decrease exponentially with the applied force. Finally, assume that the characteristic distance over which the load acts is different for the steps described by \(k_{\text{cat}}\) and \(k_b\): call this distance \(\delta_1\) for \(k_{\text{cat}}\) and \(\delta_2\) for \(k_b\). Write expressions for \(k_{\text{cat}}\) and \(k_b\) in terms of \(k_{\text{cat}}^o\) and \(k_b^o\) (the rate constants in the absence of applied force), the applied force, \(\delta_1\) and \(\delta_2\), and \(kT\) (the thermal energy of the environment).

B. (20 pts.) Assume that \(\delta_1\) is 4 nm and \(\delta_2\) is 0.4 nm. Would you expect the rate at which the enzyme takes a step to exhibit a greater force dependence when the enzyme is saturated with substrate or when the substrate concentration is very low (i.e., \(<\langle K_m \rangle\))? Explain.

C. (20 pts.) This model predicts that the observed \(K_m\) (the concentration of substrate at which the velocity is half-maximal) will also be force-dependent. If the applied force is 1 pN, \(k_{\text{cat}}^o = 0.1 \text{ s}^{-1}\), and \(k_b^o = 10^4 \text{ M}^{-1} \text{ s}^{-1}\), what will the observed \(K_m\) be? Assume that \(kT = 4.1 \text{ pN nm}\).