1. DNA Thermodynamics (25 pts)

The formation of the double-stranded DNA 20-mer oligonucleotide below from the separated single strands has been measured to proceed with $\Delta H^\circ = -128 \text{ kcal/mole}$ and $\Delta S^\circ = -346 \text{ cal/moleK}$.

Assume a total strand concentration of 3 $\mu$M (1.5 $\mu$M for each strand).

\[
T_m = \frac{\Delta H^\circ}{\Delta S^\circ + 1} = \frac{-12800 \text{ kcal/mole}}{-346 \text{ cal/moleK} \cdot 4 \ln \left(\frac{3}{2}\right)} = \frac{342 \text{ K}}{+2 \text{ or } 0} = 342 \text{ K} = 62^\circ \text{ C}
\]

(a; 7 pts) What is the $T_m$ for this oligonucleotide? What is the value of $\Delta G^\circ$ at the $T_m$?

The $T_m$ formula is:

\[
T_m = \frac{\Delta H^\circ}{\Delta S^\circ + 1}
\]

\[
\Delta G^\circ = \Delta H^\circ - T \Delta S^\circ
\]

\[
\Delta G^\circ = -128 \text{ kcal/mole} + 342 \text{ K} \cdot 346 \text{ kcal/100} \text{ molK}
\]

\[
= -128 \text{ kcal/mole} + 346 \text{ kcal/mole}
\]

\[
= -9.59 \text{ kcal/mole}
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(b) 8 pts) Briefly explain why $\Delta H^*$ and $\Delta S^*$ are each negative for the hybridization reaction.

$$\Delta H^* < 0 \text{ because this is an exothermic reaction (trivial).}$$

$$\Delta S^* < 0 \text{ because there is an ordering reaction: the conformational entropy of the DNA strands is reduced as well as the translational entropy - 2 nucleotides traveling as mr.}$$

(c) 6 pts) Briefly and qualitatively explain how the nearest-neighbor rules for prediction of DNA melting thermodynamics are analogous to the bond-energy approximation used to estimate $\Delta H^*$, for previously unknown organic compounds.

In each case, we don't have a database that

includes characterization of the millions of possible compounds. We assume that the overall

$\Delta H^* \text{ or } \Delta G^* \text{ or } \Delta S^*$ can be broken down into a

sum over individual components/interactions. For

the NN model these individual components are

counterpart stacks:

\[\text{Score for the page: } \_\_\_\_\_]
(d; 4 pts) The DNA microarrays sold by Affymetrix typically use 25-mer DNA probes. One application is to detect the amount of a known mRNA sequence in a clinical sample. Each target mRNA (or fluorescently labeled copy of same) typically hybridizes to 20 or so different probe oligonucleotides on the array. Each of these perfect match probe oligos is accompanied by a “mismatch probe” on a nearby spot, with the idea being that the target mRNA will hybridize preferentially to the target probe over the mismatch probe. One doesn’t want to operate these sensitive and expensive machines at very high temperatures. Why don’t they use sequences much longer than 25 nucleotides long on the arrays?

1. Long oligonucleotides melt at very high temperature
2. So the mismatch will be stable too - lose specificity when affinity is too great

2. Anaerobic Metabolism (25 pts)

The “Anammox” reaction (anaerobic ammonia oxidation) is carried out by bacteria in the Black Sea.

The Anammox bacteria are chemolithoautotrophs, which is considered an advantage for their possible use in wastewater treatment because they don’t have to be fed reduced carbon.

(a; 8 pts) The Anammox reaction is as follows: \( \text{NH}_4^+ (aq) + \text{NO}_2^- (aq) \rightarrow \text{N}_2 (g) + 2\text{H}_2\text{O}(l) \)

Write down separate oxidative and reductive reactions for the individual conversions of ammonium and nitrite to elemental nitrogen. The beauty part is that the electrons and protons needed for Anammox are balanced.

\[
\begin{align*}
2 \text{NH}_4^+ & \rightarrow \text{N}_2 + 6e^- + 8 \text{H}^+ \\
& \text{oxidation of ammonia} \\
(\text{a}(c3))
\end{align*}
\]

\[
\begin{align*}
2 \text{NO}_2^- & \rightarrow \text{N}_2 + 4 \text{H}_2\text{O} \\
& \text{reduction of nitrite} \\
(\text{b}(c3))
\end{align*}
\]

\[
\begin{align*}
2 \text{NH}_4^+ + 2 \text{NO}_2^- & \rightarrow 2\text{N}_2 + 4 \text{H}_2\text{O} \\
& \text{as written above}
\end{align*}
\]

(b; 4 pts) \( \Delta G^{\circ} = -357 \text{ kJ/mole} \) for the Anammox reaction. What is \( E^{\circ} \)?

\[
\begin{align*}
\Delta G^{\circ} &= -nF \theta^{\circ} \\
\theta^{\circ} &= -\frac{\Delta G^{\circ}}{nF} = -\frac{-357 \text{ kJ/mole}}{(3)(96500 \text{ C/mole}\cdot \text{mol})} \approx 1.23 \\
& \text{with 2 electrons on the above equation} \\
& \text{made here}
\end{align*}
\]

Score for the page ___________
(c) 2 pts) Is the Anammox reaction assimilative or dissimilative? Circle one.

(d) 8 pts) The source of nitrite (NO$_3^-$) for this reaction is other bugs who reduce nitrate to nitrite:

$$\text{NO}_3^- + 2 \text{H}^+ + 2 \text{e}^- \rightarrow \text{NO}_2^- + \text{H}_2\text{O}$$

This is an example of syntrophy. Briefly define syntrophy. It is obvious what the recipient in a syntrophic relationship gains. What is the advantage to the donor?

> Syntrophy is the sharing of metabolites transfer of metabolites between, among prokaryotes to their mutual benefit. Characteristic of anaerobic consortia.

The donor bug gains from the removal of a product of its electron transport chain or other metabolic process.

By LeChatelier, this will increase the thermodynamic driving force. Put another way, the donor doesn’t check its own work.

(e) 3 pts) We have discussed the metabolism of methanogens, which transfer electrons from hydrogen to carbon dioxide to produce methane. They live on the edge of starvation, because the free energy available from this reaction is low. Why don’t the methanogens just burn the methane that they make and live high on the hog?

$$\text{CH}_4 + \text{H}_2 \rightarrow 2 \text{H}_2\text{O} + 4 \text{e}^- + 4 \text{H}^+$$

There’s no oxygen! Methane is the product from the terminal electron acceptor. Under these conditions, it’s the thermodynamic minimum.

Methanogens live only where there are no electrons.
3. Redox Reactions to Live By (12 pts)

The Breathalyzer works on the principle of oxidizing ethanol to acetic acid, with concurrent reduction of orange dichromate \( \text{Cr}_2\text{O}_7^{2-} \) to green \( \text{Cr}^{3+} \).

Standard reduction potentials:

\[
\begin{align*}
(\text{a}) & \quad \text{CH}_3\text{OH} + 4 \text{H}^+ + 4 e^- \rightarrow \text{CH}_3\text{COOH} + 2 \text{H}_2\text{O} \quad E^\circ = 0.058 \text{ V} \\
(\text{b}) & \quad \text{Cr}_2\text{O}_7^{2-} + 14 \text{H}^+ + 6 e^- \rightarrow 2 \text{Cr}^{3+} + 7 \text{H}_2\text{O} \quad E^\circ = 1.33 \text{ V}
\end{align*}
\]

(a; 4 pts) What is the balanced overall reaction?

\[
\begin{align*}
3 \text{CH}_3\text{CH}_2\text{OH} + 3\text{H}_2\text{O} & \rightarrow 3 \text{CH}_3\text{COOH} + 3\text{H}^+ + 12e^- \\
2 \text{Cr}_2\text{O}_7^{2-} + 16\text{H}^+ + 12e^- & \rightarrow 4 \text{Cr}^{3+} + 8\text{H}_2\text{O}
\end{align*}
\]

\[
\begin{align*}
3 \text{CH}_3\text{CH}_2\text{OH} + 2\text{Cr}_2\text{O}_7^{2-} + 16\text{H}^+ & \rightarrow 3 \text{CH}_3\text{COOH} + 4\text{Cr}^{3+} + 11\text{H}_2\text{O}
\end{align*}
\]

(b; 2 pts) What is \( E^\circ \) for the overall reaction?

\[
E = E^\circ - 0.0592 \log Q
\]

\[
E = 1.33 - 0.0592 \log 16
\]

\[
E = 1.33 - 0.0592 \times 4
\]

\[
E = 0.95 \text{ V}
\]

(c; 6 pts) What is the actual cell voltage \( E \) if all reagents are in their chemical standard states (1 M everything) except that the pH is 4? The temperature is 25\(^\circ\)C.

\[
E = E^\circ - \frac{2.303 RT}{nF} \log Q
\]

\[
E = 1.33 - \frac{0.0592}{12} \log \left( \frac{[1]^3[1]^1}{[1]^3[1]^2(10^{-4})^{16}} \right)
\]

\[
E = 1.33 - \frac{0.0592 \times 64}{12}
\]

\[
E = 0.95 \text{ V}
\]

\[
+1 = \ln \text{ instead of log}
\]

because \( \left[ \text{reactant} \right] > \) lower than standard state concentration

Score for the page 10/10.
4. Electrochemistry (14 pts)

The Leclanché cell (misnamed the dry cell) shown at the right has a zinc anode (the negative terminal). The cathode is MnO₂ dispersed in graphite throughout the body of the cell, with the central graphite electrode acting to return the electrons from the external circuit. The chloride in the picture is a spectator ion.

(a) 10 pts The anodic half-reaction for this galvanic cell is

\[ \text{Zn}^{2+} (aq) + 2e^- \rightarrow \text{Zn} (s) \]  

The cathodic half-reaction is

\[ 2 \text{MnO}_2(s) + 2 \text{NH}_4^+ (aq) + n e^- \rightarrow 2 \text{MnO}_2(s) + 2 \text{NH}_3 (aq) + n \text{H}_2\text{O} \]  

Who is being reduced here? Calculate n for the reduction half-reaction.

Write down the overall net galvanic reaction.

\[ +3 \quad \text{Mn} \text{ is reduced, from } +4 \text{ to } +3 \]

\[ +3 \quad n = 2 \text{ as written - 2 } \text{Mn} \times 1e^- \text{ each} \]

\[ +4 \quad \text{Zn} (s) + 2 \text{MnO}_2(s) + 2 \text{NH}_4^+ (aq) \rightarrow \text{Zn}^{2+} (aq) + 2 \text{MnO}_2(s) + 2 \text{NH}_3(aq) + n \text{H}_2\text{O} \]

\[ +5 \quad \text{for wrong coefficients: } \text{MnO}_2, \text{NH}_4^+, (4) \]

\[ \text{H}_2\text{O}, \text{MnO}_2(s), (2), \text{NH}_3^+, (4) \]

if n = 4

(b) 4 pts The Leclanché cell is headed for obsolescence, in part because its voltage drops off with time.

It is being replaced by the alkaline cell with the overall reaction below, which uses the same redox couple. Why does the voltage of the Leclanché cell decline with use, whereas the voltage of the alkaline cell is constant until it is nearly dead (you need not calculate E°'s)?

\[ \text{Zn}(s) + 2 \text{MnO}_2(s) + \text{H}_2\text{O}(l) \rightarrow \text{Zn(OH)}_2(s) + 2 \text{MnO}_2(s) \]

All of the reactants and products are solids, so

H/Q does not change during the reaction. For the Leclanché cell, Zn\text{II} (aq) and NH\text{III} (aq) build up,

and cell voltage changes.

Score for the page___________
5. Kinetics (24 pts)

Kinetic partitioning describes the distribution of products for reactions under kinetic control, such as correct and incorrect base incorporation by DNA polymerases.

For the elementary reaction \( A \rightarrow B \), the differential rate law is \(-d[A]/dt = k[A]\).

(a; 3 pts) What is the integrated rate law for the first-order reaction \( A \rightarrow B \) assuming that we start with 100% \( A \) at concentration \([A]_0\), (not a trick question)?

\[
[A] = [A]_0 e^{-kt} 
\]

(b; 3 pts) Given that all of the starting material can always be found as either \( A \) or \( B \) during the reaction, derive an expression for the amount of \( B \) as a function of \( t \).

\[
[B] = \frac{[A]_0 - [A]}{[A]_0} = \frac{[A]_0 - [A]_0 e^{-kt}}{[A]_0} + 1
\]

(c; 6 pts) Now consider the scheme at the right, where \( A \) can be converted to either \( B \) or \( C \).

What are the differential rate laws for loss of \([A]\), gain of \([B]\), and gain of \([C]\)?

\[
\frac{d[A]}{dt} = \frac{k_1 [A] + k_2 [A]}{t + k_1} + k_3 [A] + k_4 [A] + k_5 [A] 
\]

\[
\frac{d[B]}{dt} = \frac{k_2 [A]}{t + k_1} + 1
\]

\[
\frac{d[C]}{dt} = \frac{k_3 [A]}{t + k_1} + 1
\]

Score for the page
(d; 3 pts) B and C are always produced at a constant ratio (in other words, for every mole of B we get \( x \) moles of C. From your answer to (c), what is \([C]/[B] = x^2\)?

\[
\frac{[C]}{[B]} = \frac{b_1}{b_2} = x + 3
\]

+1 for any ratio

(e; 3 pts) By analogy with your answer in (a) for A -> B, what is the integrated rate law for the concentration of A as a function of time?

\[
\int_0^t [A] = [A]_0 e^{-(k_1+k_2)t}
\]

+1 for any rate constant

(f; 6 pts) The total concentration of products is given by \([B] + [C] = [A]_0 - [A]\) as in (b) above. Use your answers to (d) and (e) to derive the integrated rate law for the total amount of [B] as a function of time.

\[
[C] = [A]_0 \frac{b_1}{b_2}
\]

\[
[B] + \frac{[B]}{b_1} = [A]_0 - [A]_0 e^{-(k_1+k_2)t}
\]

\[
[B] \left( 1 + \frac{b_2}{b_1} \right) = [A]_0 \left( 1 - e^{-(k_1+k_2)t} \right)
\]

\[
[B] = \left( \frac{b_2}{b_1+k_2} \right) [A]_0 \left( 1 - e^{-(k_1+k_2)t} \right)
\]

1 \[\text{overall time determined by all competing pathways.}\]
Useful Equations:

\[ \Delta S - q/T \geq 0 \]
\[ S = k \ln W \]
\[ K_a = [H^+][A^-]/[HA] \]
\[ W = N!/([I]_n!) \]
\[ n/n_0 = \exp[-(E_a - E_f)/kT] \]
\[ R = 1.987 \text{ cal/mole K} \]

\[ 2.303RT/\mathcal{F} = 0.0592 \text{ Volts at } 25^\circ C \]
\[ \mathcal{F} = 96500 \text{ Coulomb/mole} \]

Chemical standard state: 1M solutes, pure liquids, 1 atm gases

Biochemical standard state: pH 7, all species in the ionic form found at pH 7

\[ \ln K_{eq} = (-\Delta H^\circ/RT + \Delta S^\circ/R) \]
\[ [A] = [A]_0 - kt \]
\[ \ln[A] = \ln[A]_0 - kt \]
\[ 1/[A] = 1/[A]_0 + 2kt \]

\[ E = mc^2 \]
\[ PV = nRT \]
\[ e^{\alpha} + 1 = 0 \]
\[ T_m = \Delta H^\circ/\Delta S^\circ \cdot \sqrt{\frac{R}{n\mathcal{F}} / \text{C mole}^2} \]
\[ E = E^\circ - 2.303(RT/\mathcal{F}) \log_{10} Q \]
\[ \Delta G = -nFE \]

\[ a^2 + b^2 - 2ab\cos C = c^2 \]

This is the only section you needed.