You have 53 minutes for this exam.

Exams written in pencil or erasable ink will not be re-graded under any circumstances.

Explanations should be <u>concise</u> and <u>clear</u>. I have given you more space than you should need. There is extra space on the last page if you need it.

You will need a calculator for this exam. No other study aids or materials are permitted.

Generous partial credit will be given, *i.e.*, if you don't know, guess.

Many useful equations are given on page 9.

<u>Honor Pledge: At the end of the examination time , please write out the following sentence and sign it, or talk to me about it:</u>

"I pledge on my honor that I have not given or received any unauthorized assistance on this examination."

(82)

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 $\Delta H° = -128$ kcal/mole and $\Delta S° = -346$ cal/moleK. Assume a total strand concentration of 3 μM (1.5 μM for each strand).

$$5'-TATTAAGCGACCACACATAA$$
$$ATAATTCGCTGGTGTGTATT-5'$$
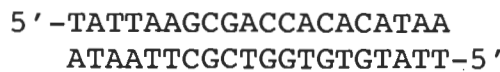
(a; 7 pts) What is the T_m for this oligonucleotide? What is the value of $\Delta G°$ at the T_m?

$$T_m = \frac{\Delta H°}{\Delta S° + R \ln(C_T/4)} = \frac{-128000 \text{ cal/mole}}{-346 \text{ cal/mole K} + 1.987 \ln(3\times10^{-6}/4)} = 342 K$$
$$(= 69°C)$$

wrong but uses ... (or: 402 K ...)

Was ⊖ on page 9 - ouch!

$$\Delta G_{T_m} = \Delta H° - T\Delta S° = -128 \text{ kcal/mole} + 342 K \cdot \frac{346 \text{ kcal}}{1000 \text{ mole K}}$$

+1 for consistent substitution

full credit for consistently using the incorrect T_m formula

$$= -9.59 \text{ kcal/mole}$$

[or +11.28 kcal/mole - wrong but consistent]

(b; 8 pts) Briefly explain why ΔH° and ΔS° are each negative for the hybridization reaction.

+3 for either,
+4 for (✗✗) both

$\Delta H° < 0$ because this is an exothermic reaction [+1 for just this] (tautology).

Hydrogen bond formation and stacking interactions are exothermic — stacking → hypochromicity as well.

+3 for either,
+4 for both

$\Delta S° < 0$ because this is an ordering reaction [+2 for just this] — the conformational entropy of the DNA strands is reduced as well as the translational entropy — 2 molecules traveling as one.

(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 ΔH°_f for previously unknown organic compounds.

+2

+2

+2

In each case we don't have a database that includes characterization of the millions of possible compounds. We assume that the overall $\Delta H°$ or $\Delta G°$ or $\Delta S°$ can be broken down into a sum over individual components/interactions. For the NN model these individual components are dinucleotide stacks:

Score for the page_____
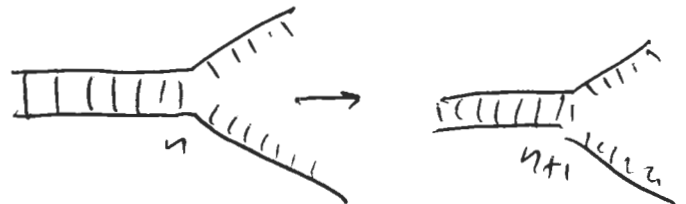
(d; 4 pts) The DNA microarrays sold by Affymetrix typically use 25-mer DNA probes. One application is to detect the amount of a known mRNA sequence in a clinical sample. Each target mRNA (or fluorescently labeled copy of same) typically hybridizes to 20 or so different probe oligonucleotides on the array. 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?

+2 Long oligonucleotides melt at very high temperature

+2 So the mismatch will be stable too - lose specificity when affinity is too great
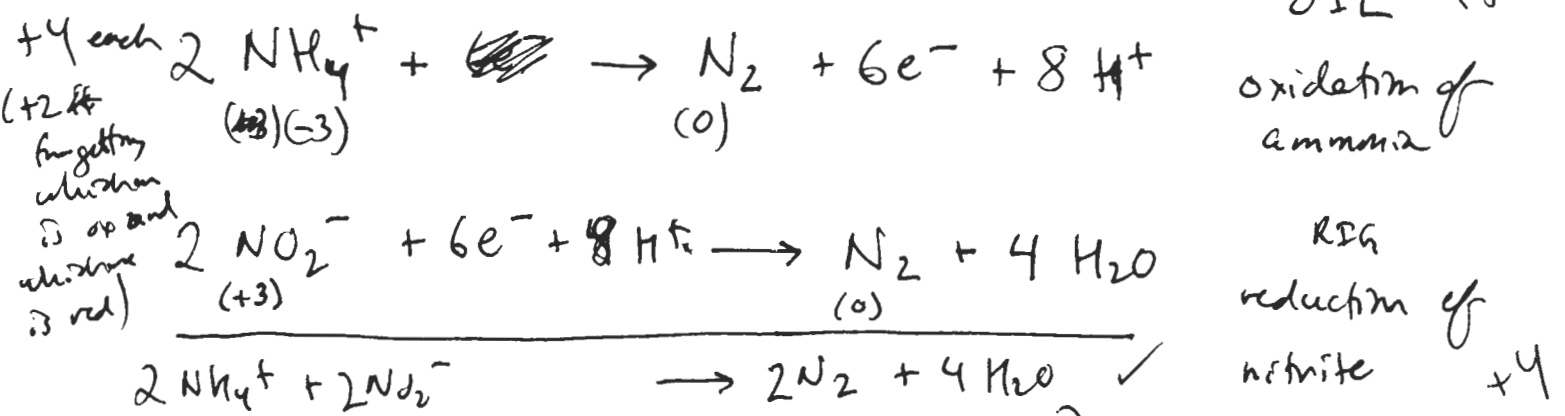
2. Anaerobic Metabolism (25 pts)

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: $NH_4^+(aq) + NO_2^-(aq) \rightarrow N_2(g) + 2H_2O(l)$

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.

OIL   ×8

oxidation of ammonia

+4 each (+2 for getting oxidation & whatever is ox and whatever is red)

$2 NH_4^+ + \cancel{} \rightarrow N_2 + 6e^- + 8 H^+$
   (+3)(-3)   (0)

RIG

reduction of nitrite   ×4

$2 NO_2^- + 6e^- + 8 H^+ \rightarrow N_2 + 4 H_2O$
   (+3)   (0)

$2 NH_4^+ + 2 NO_2^- \rightarrow 2 N_2 + 4 H_2O$ ✓

(b; 4 pts) $\Delta G^{\circ\prime} = -357$ kJ/mole for the Anammox reaction. What is $E^{\circ\prime}$? → 3 electrons as written above needed here (as written above)

+1 ($\Delta G^{\circ\prime} = -n F E^{\circ\prime}$

$E^{\circ\prime} = -\dfrac{\Delta G^{\circ\prime}}{nF} = -\dfrac{357 \text{ kJ/mole}}{(3) \, 96500 \, C/mole}$ = 1.23 $\dfrac{J}{C}$ = 1.23 V

+1   +2

Score for the page_____

(c; 2 pts) Is the Anammox reaction assimilative or (dissimilative?)(Circle one.)   +2

(d; 8 pts) The source of nitrite (NO_2^-) for this reaction is other bugs who reduce nitrate to nitrite:

$$NO_3^- + 2\,H^+ + 2\,e^- \rightarrow NO_2^- + H_2O$$
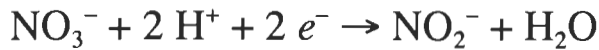
This is an example of syntrophy. Briefly define syntrophy. It is obvious what the recipient in a syntrophic relationship gains. What is the advantage to the donor?

(+3) 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 the (+2) product of (+8) its electron transport chain or other metabolic process.

(+3) By LeChatelier, this will increase the thermodynamic driving force. Put another way, the donor doesn't choke in 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?

+2 +3 or +2 for long explanation.

There's no oxygen! Methane is the product from the terminal electron acceptor — under those conditions, it's the thermodynamic minimum
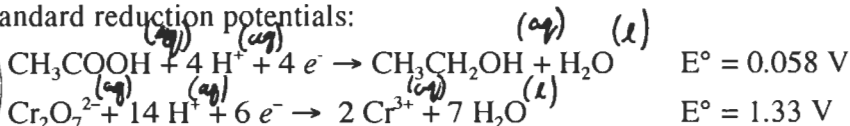
Methanogens live only where there are no aerobes

Score for the page_____

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 $Cr_2O_7^{2-}$ to green Cr^{3+}.
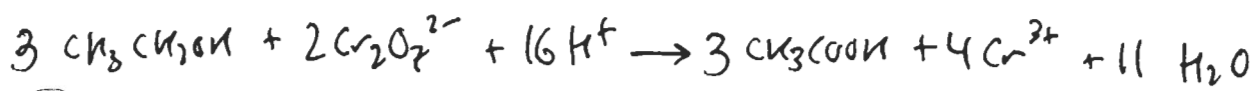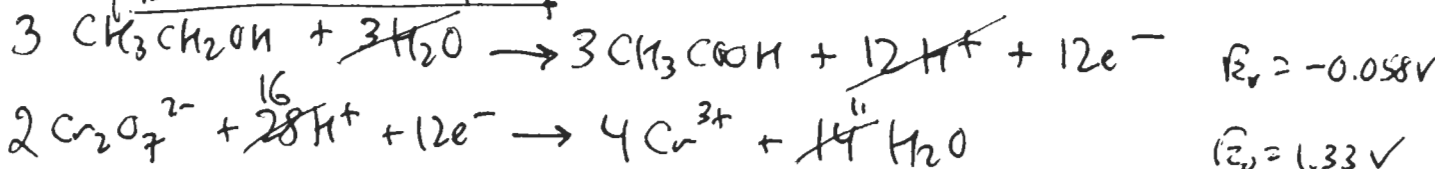
Standard reduction potentials:

reverse (×3) $CH_3COOH + 4 H^+ + 4 e^- \rightarrow CH_3CH_2OH + H_2O$ $E° = 0.058$ V

(×2) $Cr_2O_7^{2-} + 14 H^+ + 6 e^- \rightarrow 2 Cr^{3+} + 7 H_2O$ $E° = 1.33$ V

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

→ Balance the electrons

$3 CH_3CH_2OH + 3H_2O \rightarrow 3 CH_3COOH + 12 H^+ + 12 e^-$ $E_r = -0.058V$

$2 Cr_2O_7^{2-} + 28 H^+ + 12 e^- \rightarrow 4 Cr^{3+} + 14 H_2O$ $E_o = 1.33 \checkmark$

$3 CH_3CH_2OH + 2 Cr_2O_7^{2-} + 16 H^+ \rightarrow 3 CH_3COOH + 4 Cr^{3+} + 11 H_2O$

**(b; 2 pts) What is E° for the overall reaction?** $E_G = 1.33 - 0.06 V = 1.27V$

(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°C.

$$E = E° - \frac{2.303 \, RT}{nF} \log Q$$

$$= 1.27 - \frac{0.0592}{12} \log \left(\frac{[1]^3 [1]^4}{[1]^3 [1]^2 (10^{-4})^{16}} \right)$$

$\frac{1}{10^{-64}} = 10^{64}$

+5 total for everything correct except biochemical standard state

$$= 1.27 - \frac{0.0592 \cdot 64}{12}$$

$$= 0.95 V$$

< E° ✓ because [reactant] is lower than standard state concentration
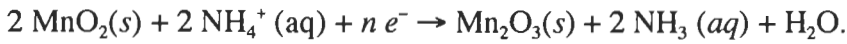
+1 ⇒ ln instead of log

Score for the page_____

4. **Electrochemistry (14 pts)**   (Stolen from Mc Murry)

The Leclanché cell (misnamed the dry cell) shown at the right has a
zinc anode (the negative terminal). The cathode is MnO_2 dispersed
in graphite throughout the body of the cell, with the central graphite
electrode acting to return the electrons from the external circuit.
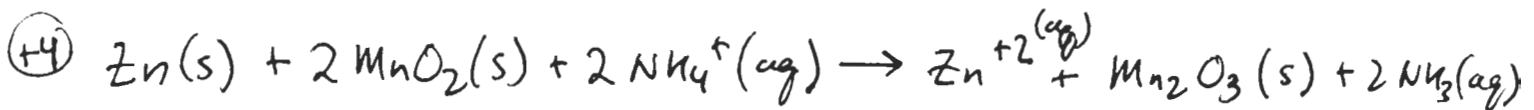The chloride in the picture is a spectator ion.
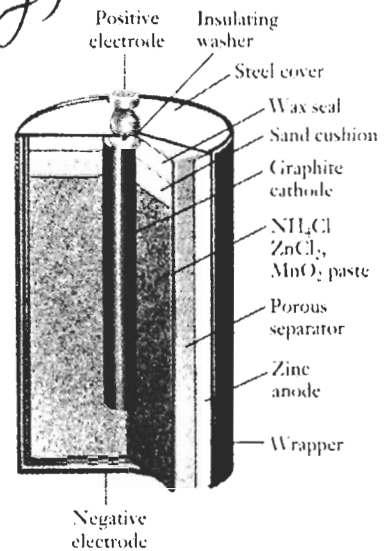
(a; 10 pts) The anodic half-reaction for this galvanic cell is
$Zn(s) \rightarrow Zn^{2+} (aq) + 2\ e^-$. The cathodic half-reaction is

$2\ MnO_2(s) + 2\ NH_4^+ (aq) + n\ e^- \rightarrow Mn_2O_3(s) + 2\ NH_3 (aq) + H_2O.$

Who is being reduced here? Calculate n for the reduction half-reaction.
Write down the overall net galvanic reaction.

(+3) Mn is reduced, from +4 to +3

(+3) n = 2 as written - 2 Mn × 1e⁻ each

(+4) $Zn(s) + 2\ MnO_2(s) + 2\ NH_4^+(aq) \rightarrow Zn^{+2}_{(aq)} + Mn_2O_3(s) + 2\ NH_3(aq)$
$+ H_2O$

+3 for wrong coefficients for MnO_2, NH_4^+, (4)
H_2O, Mn_2O_3 (2), 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)?

$Zn(s) + 2\ MnO_2(s) + H_2O(l) \rightarrow Zn(OH)_2(s) + Mn_2O_3(s)$

+1 +2
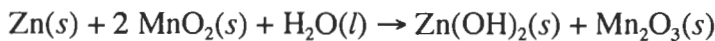
All of the reactants and products are solids, so
+1. Q does not change during the reaction. For the
Le lanché cell, $Zn^{+2}(aq)$ and $NH_3(aq)$ build up,
+2| and cell voltage changes.

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 -> B, the differential rate law is $-d[A]/dt = k[A]$.

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

$$+3 \qquad [A] = [A]_0\, e^{-kt}$$

(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 time.

$$[B] = [A]_0 - [A] = [A_0] - [A]_0\, e^{-kt} \quad +3$$

$$+2$$

$$[\text{This question was asked in class...}] = [A_0](1 - e^{-kt}) \quad +1$$

there are not first = order vs. 2nd order!

(c; 6 pts) Now consider the scheme at the right, where A can be converted to either B or C.   ← *of elementary reactions*
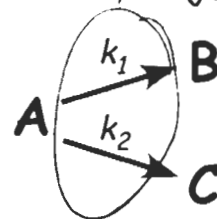
What are the differential rate laws for loss of [A], gain of [B], and gain of [C]?

$$+2 \quad (-)\frac{d[A]}{dt} = k_1[A] + k_2[A] = (k_1 + k_2)[A] \quad +3$$

$$+\frac{d[B]}{dt} = k_1[A] \quad +1$$

$$\frac{d[C]}{dt} = k_2[A] \quad +1$$

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 $[C]/[B] = x$?

$$\frac{[C]}{[B]} = \frac{k_2}{k_1} = x \quad +3$$

+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?

$+3$

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

+1 for any related answer

$k_1 + k_2$ is just a 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.

$$[C] = [B] \frac{k_2}{k_1}$$

(+3) the idea of subbing for [C]

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

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

(+3)

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

kinetic partitioning

overall $t_{1/2}$ determined by all competing pathways.

Page	Score
1	
2	
3	
4	
5	
6	
7	
8	
Total	

Score for the page_____

Useful Equations:

$\Delta S - q/T \geq 0$

$S = k \ln W$

$K_a = [H^+][A^-]/[HA]$

$W = N!/(\prod n_i!)$

$^\circ C = ^\circ K - 273.15$

$pH = -\log([H^+])$

$\Delta G = \Delta H - T\Delta S$

$\Delta G^\circ = -RT \ln K_{eq}$

$n_i/n_0 = \exp[-(E_i-E_0)/kT]$

R = 1.987 cal/mole K

$E = mc^2$

$PV = nRT$

$e^{i\pi} + 1 = 0$

$T_m = \Delta H^\circ/[\Delta S^\circ + R\ln(C_t/4)]$

$E = E^\circ - 2.303(RT/n\mathcal{F})\log_{10}Q$

D'OH

$2.303RT/\mathcal{F} = 0.0592$ Volts at 25 $^\circ$C

$\mathcal{F} = 96500$ C(oulomb)/mole 1 V = 1 J/C

$\Delta G = -n\mathcal{F}E$

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

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

$\ln K_{eq} = (-\Delta H^\circ/RT + \Delta S^\circ/R)$

$[A] = [A]_0 - kt$

$\ln[A] = \ln[A]_0 - kt$

$1/[A] = 1/[A]_0 + 2kt$

this is the only R you needed

Score for the page_____