You have 53 minutes for this exam.
Explanations should be concise and clear. There is lot of 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.

Useful Equations:

\[
\sigma_Y^2 = \lim_{N \to \infty} \frac{1}{N} \left[ \sum_i (Y_i - \bar{Y})^2 \right] \quad \text{pH} = - \log([H^+]) \quad \sigma_Y^2 = \left( \frac{\partial Y}{\partial u} \right)^2 \sigma_u^2 + \left( \frac{\partial Y}{\partial v} \right)^2 \sigma_v^2 + \ldots
\]

Thus,

For \( Y = au + bv \), \( \sigma_Y = \sqrt{a^2 \sigma_u^2 + b^2 \sigma_v^2} \).
For \( Y = \frac{au}{bv} \), \( \sigma_Y = \sqrt{\frac{\sigma_u^2}{u^2} + \frac{\sigma_v^2}{v^2}} \).

\( R = 0.08206 \text{ L} \cdot \text{atm/mole K} \) \quad \text{\( T^2 = 4\pi^2 a^3/GM \) \quad \ln K_{eq} = -\Delta H^\circ/(RT) + \Delta S^\circ/R \)}

\( R = 8.314 \text{ J/mole K} = 1.987 \text{ cal/mole K} = N_A k_B \) \quad \text{\( SEM = \frac{\sigma}{\sqrt{n}} \)}

\( N_A = 6.02 \times 10^{23} \text{ particles/mole} \) \quad \text{zepto-atto-femto-pico-nano-micro-milli-centi-deci-base}

\( ^\circ C = ^\circ K - 273.15 \) \quad \( P(v)dv = Cv^2 \exp(-mv^2/2kT) \) \ln \( k = (-E_a/RT) + \ln A \)

\( \text{pH} = pK_a + \log([A^-]/[HA]) \) \quad \( K_p = K_c (RT)^\Delta n \) \quad \( K_w = [H^+][OH^-] = 10^{-14} \)

\( \text{Absorbance} = \varepsilon c \ell = \log(I_0/I) \) \quad \( PV = nRT \)

\( pK_a = - \log(K_a) \) \quad \( \text{pH(e.p.)} = \frac{1}{2} (pK_{a1} + pK_{a2}) \)

\( \left[ -\frac{\hbar^2}{2\mu} \nabla^2 + V(r) \right] \Psi(r) = E \Psi(r) \)

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

“I pledge on my honor that I have not given or received any unauthorized assistance on this examination.”
1. **Absorbance and Fluorescence (40 pts):**
(a; 5 pts) Sketch the two different geometries for excitation and observed light for absorbance vs. fluorescence spectrometers.

(b; 3 pts) From the definition of how we measure absorbance, how do we know it must be unitless?

(c; 6 pts) From Beer’s law, show that the units of the molar extinction coefficient must be M$^{-1}$cm$^{-1}$.

(d; 9 pts) The molar extinction coefficient of ATP is 15400 M$^{-1}$cm$^{-1}$. Convert this value into an area per molecule, and compare this value to the physical area of the ATP chromophore shown. Approximate the length of all bonds as 0.1 nm, the area of a hexagon as the area of the corresponding circle ($\pi r^2$), and the area of each ring as identical. What do you conclude?
(e, 5 pts) For our protein lab we used an extinction coefficient in terms of micrograms per ml instead of a molar extinction coefficient. Why was the different unit appropriate?

(f, 12 pts) Recall that fluorescence is a competition between loss of energy through emission of a photon vs. through any other means. In general, are most colored molecules fluorescent in the visible/IR or not? Give two possible reasons for the enhanced fluorescence of ethidium bromide when it is bound to DNA vs. free in solution, where it has more conformational freedom and is completely accessible to solvent. (I will be very surprised if anyone gives the answer accepted in the literature: this is about thinking.)

(From https://www.hoelzel-biotech.com/en/infothek/nucleic-acid-detection/)
2.0 ± 0.3  Accuracy, precision, error analysis (25.0000 ± 0.0001 pts):
(a; 20 pts) If you mix 2.57 ± 0.03 mL of a 1.523 M NaCl stock solution with 7.43 ± 0.04 mL of water, what is the final salt concentration? Give your reasoning for the error you impute to the stock solution [NaCl], and provide a final answer to the appropriate number of sig figs ± propagated uncertainty to one sig fig.

(b; 5 pts) Let’s say that someone measures the chloride ion concentration of the diluted solution with an extremely precise method like atomic absorption to be 0.457321 M. Is the error in your concentration likely to be a systematic error or a random error? Speculate on what could cause it.
3. Plotting data (10 pts):
(a; 10 pts) Sketch a van’t Hoff plot (based on \( \ln K = (\Delta H^\circ - T\Delta S^\circ)/RT \)) for an equilibrium with \( \Delta H^\circ < 0 \) and \( \Delta S^\circ > 0 \).

4. Random lab questions (25 pts)
(a; 5 pts) What was the purpose of the phenanthroline in the Fe/egg lab?

(b; 8 pts) If you run an enzymatic reaction until all the concentrations stops changing and then add additional enzyme, explain why there should be no further change if everything is going well. If something does happen, give a possible explanation.
(c; 4 pts) What was the basis of our measurement of acetaldehyde concentration in the alcohol dehydrogenase lab? (EtOH + NAD$^+$ ⇌ acetaldehyde + NADH)

(d; 8 pts) Explain briefly why fluorescence is to the red of absorbance for any given molecule.