COOPERATIVE LIGAND BINDING: OXYGEN BINDING TO HEMOGLOBIN

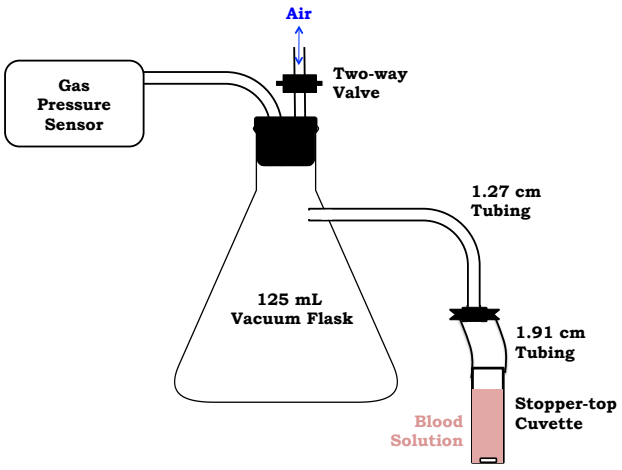
Lab Activity

1. Create 3 mL of a 1:200 dilution of bovine blood with 50 mM phosphate buffer (pH 7.4) in a small glass test tube.

2. Blank the spectrophotometer with 50 mM phosphate buffer (pH 7.4).

3. Discard the buffer in the cuvette, and transfer the diluted blood into the cuvette.
4. Add a small magnetic stir bar.

5. Connect the cuvette to the variable-pressure apparatus:



6. Record atmospheric gas pressure in mm Hg.

7. Record the current room temperature.

8. Through the two-way valve, pull the vacuum to 35 mm Hg, being careful not to let the contents of the cuvette boil. Close the two-way valve.

9. Position the cuvette on a magnetic stir plate and gently stir the contents of the cuvette for 30 minutes.

10. Position the cuvette in the spectrophotometer and read the absorbance between 520 and 590 nm.

11. Increase the pressure inside the variable-pressure cuvette apparatus. To add air in a controlled manner, fill a 20 mL syringe with a volume of 10 mL air and attach the syringe to the two-way value. Open the value, allowing the air to enter the system. Close the value, and remove the syringe. Record the new gas pressure.

12. Position the cuvette on a magnetic stir plate and gently stir the contents of the cuvette for 2 minutes.

13. Position the cuvette in the spectrophotometer and read the absorbance between 520 and 590 nm.

14. Repeat steps 11-13 until the gas pressure inside the system has returned to atmospheric pressure.

Data Analysis

1. Determine the pO_2 present in the system from the absolute pressure of air within the apparatus ($P_{observed}$) for each observed pressure reading:

$$pO_2 = 0.21\left(P_{observed} - pH_2O\right)$$

Temperature (°C)	pH_2O (mm Hg)
18	15.5
19	16.5
20	17.5
21	18.7
22	19.8

2. Make a plot of $A_{541\ nm}$ versus pO_2. Is hemoglobin behaving non-cooperatively or cooperatively?

3. Use non-linear regression with Microsoft Excel to solve for the baseline, amplitude, p50, and h parameters of the Hill model for your data at $A_{541\ nm}$:

$$A_{observed} = \text{baseline} + \text{amplitude}\left(\frac{\left(pO_2\right)^h}{\left(P_{50}\right)^h + \left(pO_2\right)^h}\right)$$

4. What are the standard deviations associated with each parameter?

5. Add the calculated $A_{541\ nm}$ values to the plot created for Question #2 to compare the experimental data to the best-fit curve.