1. (20 points)
The kinetics of an enzyme were analyzed in both the absence and presence of **Inhibitor A** and **Inhibitor B**. Given the following data, calculate or construct the following for A and B on separate graphs.

   a) Plot the data as a Michaelis-Menten saturation curve.

   b) Estimate the $K_m$ and $V_{max}$ from these curves both in the presence and absence of inhibitors.

   c) Plot the data in the Lineweaver-Burk format
      *Make sure to label the both the inhibitor line and the no inhibitor line

   d) **Mathematically** determine the $K_m$ and $V_{max}$.

   e) What types of inhibitors are A and B? How can you tell?

   f) On each graph draw a line that would indicate an increase in the concentration of inhibitor.

   *Make sure to turn in all your plots, corresponding equations and calculations.

<table>
<thead>
<tr>
<th>[S] (mM)</th>
<th>V (mmol/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Inhibitor</td>
</tr>
<tr>
<td>.2</td>
<td>5.0</td>
</tr>
<tr>
<td>.4</td>
<td>7.5</td>
</tr>
<tr>
<td>.8</td>
<td>10.0</td>
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<td>4.0</td>
<td>13.6</td>
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<td>12.5</td>
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<tr>
<td>4.0</td>
<td>13.6</td>
</tr>
</tbody>
</table>

2. (15 points) Draw the following disaccharides in Haworth projections. **Label** the anomic carbon with a * and **circle** any reducing ends.

   a) galactose β(1,4) mannose
   b) glucose β,α(1,1) galactose
   c) fructose α(2,5) ribose
<table>
<thead>
<tr>
<th>[S] (mM)</th>
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<th>A (5mM)</th>
</tr>
</thead>
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<td>7.5</td>
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<td>0.8</td>
<td>10</td>
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</tr>
<tr>
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<td>10.7</td>
<td>8.3</td>
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<tr>
<td>2</td>
<td>12.5</td>
<td>10.7</td>
</tr>
<tr>
<td>4</td>
<td>13.6</td>
<td>12.5</td>
</tr>
</tbody>
</table>

Estimated $K_m$ without I: $\sim 0.4$ mM

Estimated $K_m$ with I: $\sim 0.7$ mM

$V_{max}$ without I: $\sim 15$ mmol/min

$V_{max}$ with I: $\sim 14$ mmol/min
<table>
<thead>
<tr>
<th>[S] (mM)</th>
<th>No Inhibitor</th>
<th>A (5mM)</th>
<th>1/[S]</th>
<th>1/V (no I)</th>
<th>1/V (+A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>5</td>
<td>3</td>
<td>5</td>
<td>0.2</td>
<td>0.3333333333</td>
</tr>
<tr>
<td>0.4</td>
<td>7.5</td>
<td>5</td>
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<td>0.1333333333</td>
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<tr>
<td>0.8</td>
<td>10</td>
<td>7.5</td>
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<td>8.3</td>
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<td>10.7</td>
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<tr>
<td>4</td>
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<td>12.5</td>
<td>0.25</td>
<td>0.073529412</td>
<td>0.08</td>
</tr>
</tbody>
</table>

**Graphical Analysis**

For the reaction $V = \frac{1}{[S]} \left( \frac{1}{[M]} \right)$, where $V$ is the rate of reaction in mmol/min, $[S]$ is the substrate concentration in mM, and $[M]$ is the inhibitor concentration in mM.

- **No Inhibitor**: $y = 0.0266x + 0.0668$
  
  $V_{max} = \frac{1}{0.0668} = 15.0 \text{ mmol/min}$
  
  $K_m = \frac{0.0266}{15} = 0.4 \text{ mM} = K_m$

- **With Inhibitor**: $y = 0.0533x + 0.0668$
  
  $V_{max} = \frac{1}{0.0668} = 15.0 \text{ mmol/min}$
  
  $K_m = \frac{0.0533}{15} = 0.0355 \text{ mM} = 0.8 \text{ mM}$

**Graph Notes**

- The graph shows two lines, one for $1/V$ (no inhibitor) and another for $1/V$ (inhibitor).
- The line for $1/V$ (no inhibitor) crosses the y-axis at $y = 0.0668$.
- The line for $1/V$ (inhibitor) crosses the y-axis at $y = 0.0668$.
- The slope of the line for $1/V$ (inhibitor) is $0.0533$.
- The slope of the line for $1/V$ (no inhibitor) is $0.0266$.

**Inhibitor Type**

- The inhibitor is competitive.
- $K_m$ changes, but $V_{max}$ stays same.

**Graph Interpretation**

- The graph shows the relationship between the reaction rate ($V$) and the substrate concentration ($[S]$) with and without the inhibitor.
- The y-intercept for the no-inhibitor line is $y = 0.0668$,
- The y-intercept for the inhibitor line is also $y = 0.0668$.
- The slope of the line for the inhibitor is $0.0533$.
- The slope of the line for the no-inhibitor is $0.0266$.

**Equations**

- No Inhibitor: $y = 0.0266x + 0.0668$
- With Inhibitor: $y = 0.0533x + 0.0668$
<table>
<thead>
<tr>
<th>[S] (mM)</th>
<th>No Inhibitor</th>
<th>B (0.1mM)</th>
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<tbody>
<tr>
<td>0</td>
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<tr>
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Inhibitor B

Michaelis-Menten

Estimated $K_m$ without $I$ ~0.3-0.4 mM with $I$ 0.3-0.4 mM

$V_{max}$ ~14 mmol/min ~6 mmol/min
<table>
<thead>
<tr>
<th>[S] (mM)</th>
<th>No Inhibitor</th>
<th>B (0.1 mM)</th>
<th>1/[S]</th>
<th>1/V (no I)</th>
<th>1/V (+B)</th>
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<tbody>
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</tr>
</tbody>
</table>

No inhibitor: \( y = 0.266x + 0.0668 \)

\( V_{max} = \frac{1}{0.0668} = 15 \text{ mmol/min} \)

\( K_m = \frac{0.0266}{15} = 0.017777778 = K_m = 0.4 \text{ mM} \)

+ Inhibitor: \( y = 0.0668x + 0.166 \)

\( V_{max} = \frac{1}{0.166} = 6.0 \text{ mmol/min} \)

\( K_m = \frac{0.0668}{6.0} = 0.011133333 = K_m = 0.4 \text{ mM} \)
2a. galactose $\beta(1,4)$ mannose

[diagram of galactose and mannose with chemical structures]

Either up or down on mannose

b. glucose $\beta,\alpha(1,1)$ galactose

[diagram of glucose and galactose with chemical structures]

c. either $\alpha$ or $\beta$ ribose

[diagram of ribose with chemical structures]