I. Identification of control reactions in a pathway

A. Where is the regulatory reaction?
   2. Early in the sequence or pathway (A → B, B → C).
   3. At branch points (B → G, B → H).
   4. At steps where the reverse reaction is catalyzed by a different enzyme (B ←→ C).

B. Kinetic considerations
   1. Maximal reaction rate (i.e., number of binding sites), \( V_{\text{max}} \)
   2. Affinity of the enzyme for its substrate, \( K_m \) (or \( K_{s,5} \))
   3. Equilibrium constants and mass action ratios
   4. Allosteric activators and inhibitors

C. Possible control reactions and respective controls
   1. Hexokinase (A → B) G-6-P (–)
   2. Glycogen synthetase (B → G) G-6-P (+)
   3. Glycogen phosphorylase AMP, Ca\(^{++}\) (+), ATP (–)
   4. 6-Phosphofructokinase ATP + citrate (–)), overcome by F-6-P, AMP, P, 6-PG, F-2,6-P\(_2\) (+)
   5. Glyceraldehyde-3-P-DH NAD/NADH ratio
   6. Pyruvate kinase F-1,6-P\(_2\), 6-PG, F-2,6- P\(_2\) (+), ATP (–)
D. Equilibrium constants (K\text{eq}) and Mass Action Ratio (MAR)

1. K\text{eq} is measured under set conditions of concentration, temperature, and pressure.
2. MAR is calculated from actual intracellular concentrations of reactants and products.
   
   e.g., F-6-P + ATP $\rightarrow$ F-1,6-P$_2$ + ADP

\[
K_{eq} = \frac{[F-1,6-P_2] \times [ADP]}{[F-6-P] \times [ATP]} \quad \text{under set conditions}
\]

and

\[
\text{MAR} = \frac{F-1,6-P_2 \times [ADP]}{[F-6-P] \times [ATP]} \quad \text{actual cellular conditions}
\]

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Activities</th>
<th>K\text{eq}</th>
<th>MAR</th>
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</thead>
<tbody>
<tr>
<td>Hexokinase</td>
<td>1.5</td>
<td>4,000</td>
<td>0.08</td>
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<tr>
<td>Phosphoglucoisomerase</td>
<td>176</td>
<td>0.4</td>
<td>0.24</td>
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<tr>
<td>6-Phosphofructokinase</td>
<td>56</td>
<td>1,000</td>
<td>0.03</td>
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<tr>
<td>Aldolase</td>
<td>78</td>
<td>0.0001</td>
<td>0.00001</td>
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<tr>
<td>Triosephosphate isomerase</td>
<td>2,650</td>
<td>0.04</td>
<td>0.24</td>
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<td>Glyceraldehyde-3-phosphate dehydrogenase plus phosphoglycerate kinase</td>
<td>440/169</td>
<td>1,000</td>
<td>9</td>
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<tr>
<td>Phosphoglycerate mutase</td>
<td>100</td>
<td>0.1</td>
<td>0.12</td>
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<tr>
<td>Enolase</td>
<td>158</td>
<td>3.5</td>
<td>1.4</td>
</tr>
<tr>
<td>Pyruvate kinase</td>
<td>387</td>
<td>2-20,000</td>
<td>40</td>
</tr>
</tbody>
</table>

II. Enzyme kinetics

A. Reaction rates
   1. Zero order
   2. First order
   3. Mixed order

B. Effect of substrate concentration
   1. Michaelis-Menton hypothesis
   2. Significance of K\text{m}
   3. Relationship of K\text{m}, substrate concentration, and reaction order
III. Other kinetics

A. Sigmoidal kinetics -- $K_s$
   1. Indicates cooperativity
   2. Can be caused by allosteric effectors, pH, salts

B. Allosteric effectors
   1. Inhibitors
   2. Activators
   3. Allows decision making between pathways.

IV. Regulation of cellular processes

A. Change in amount of enzyme
   1. Adaptive vs constitutive
   2. Time required -- slow

B. Phosphorylation of enzymes
   1. Glycogen metabolism
   2. Lipid metabolism
   3. Fast
[Substrate] (mM)