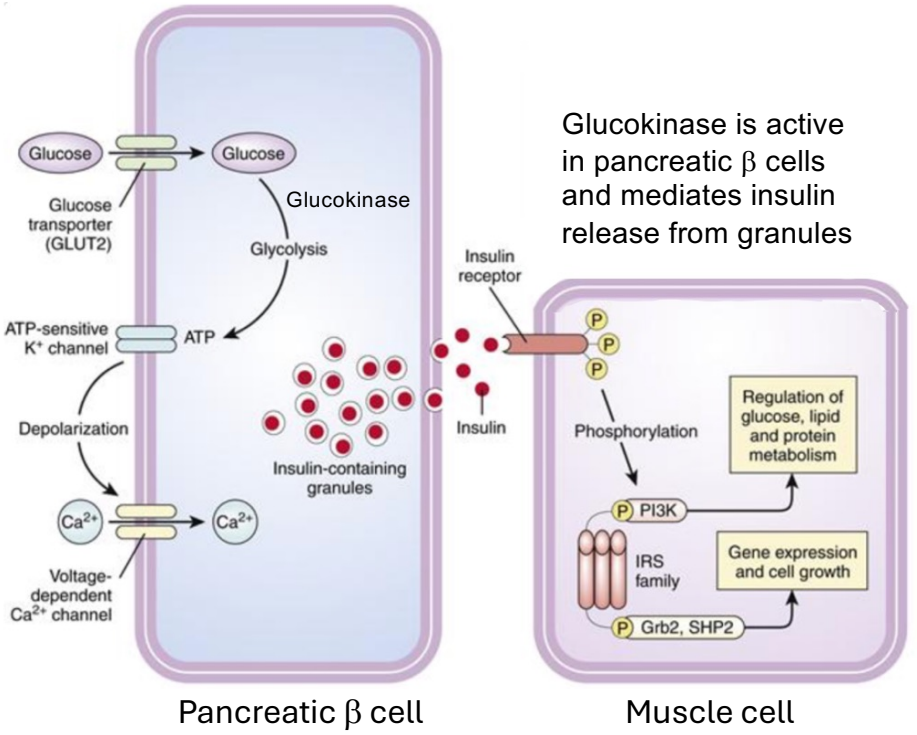
BIOC 384: M09.T06-Miesfeld

Assigned Reading: *Biochemistry* Chapter 12.4ab

Regulation of Metabolic Flux in Glycolysis

The Big Picture

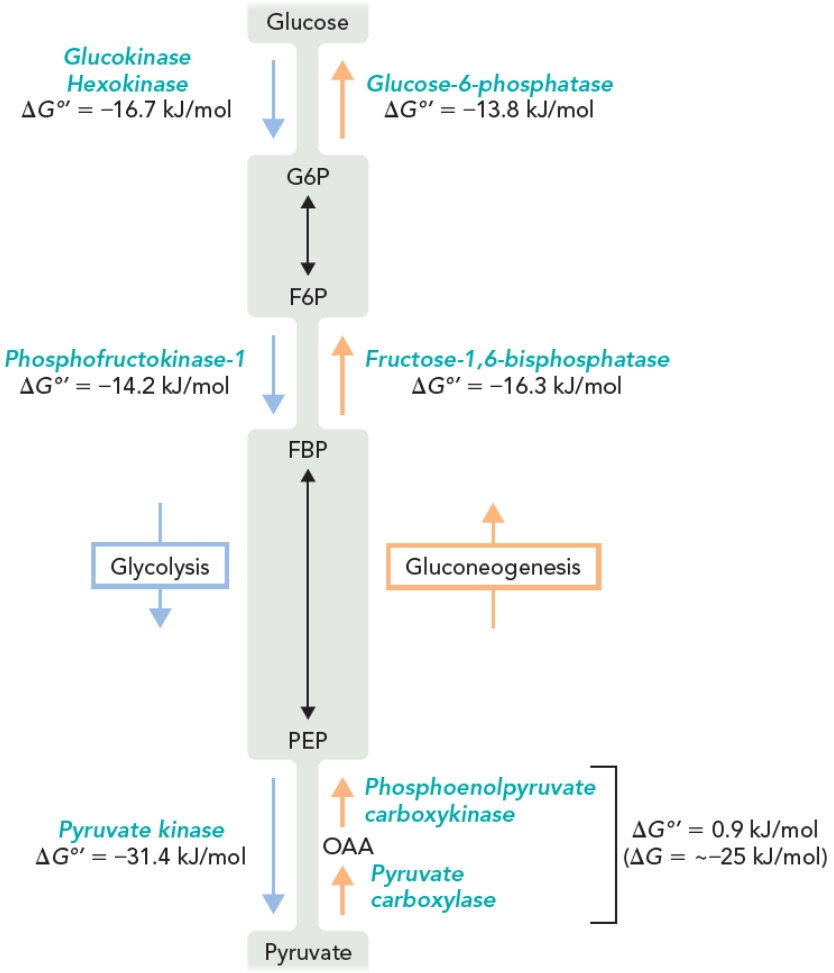
- Regulation of glycolysis ensures that ATP production is matched to cellular energy needs through control of enzyme activity and substrate availability.
- Irreversible steps catalyzed by hexokinase, glucokinase, phosphofructokinase-1, and pyruvate kinase function as metabolic control points to direct pathway flux efficiently.



Glucokinase is active in pancreatic β cells and mediates insulin release from granules

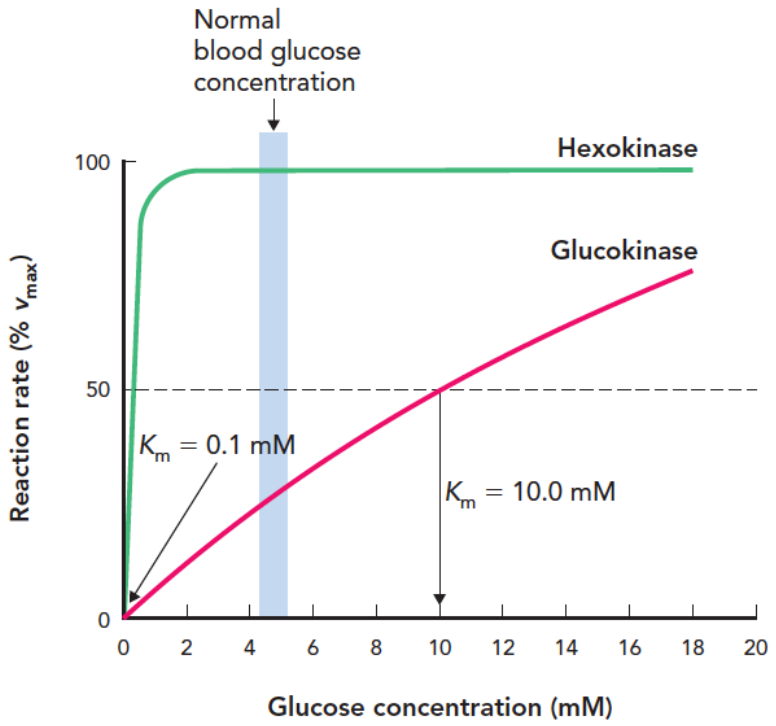
Control of Metabolic Flux

- Opposing pathways such as glycolysis and gluconeogenesis share reversible steps but use distinct enzymes at irreversible bottlenecks to control direction of flux.
- These rate-limiting enzymes act as regulated valves, ensuring glycolysis produces ATP when needed and gluconeogenesis synthesizes glucose during fasting.



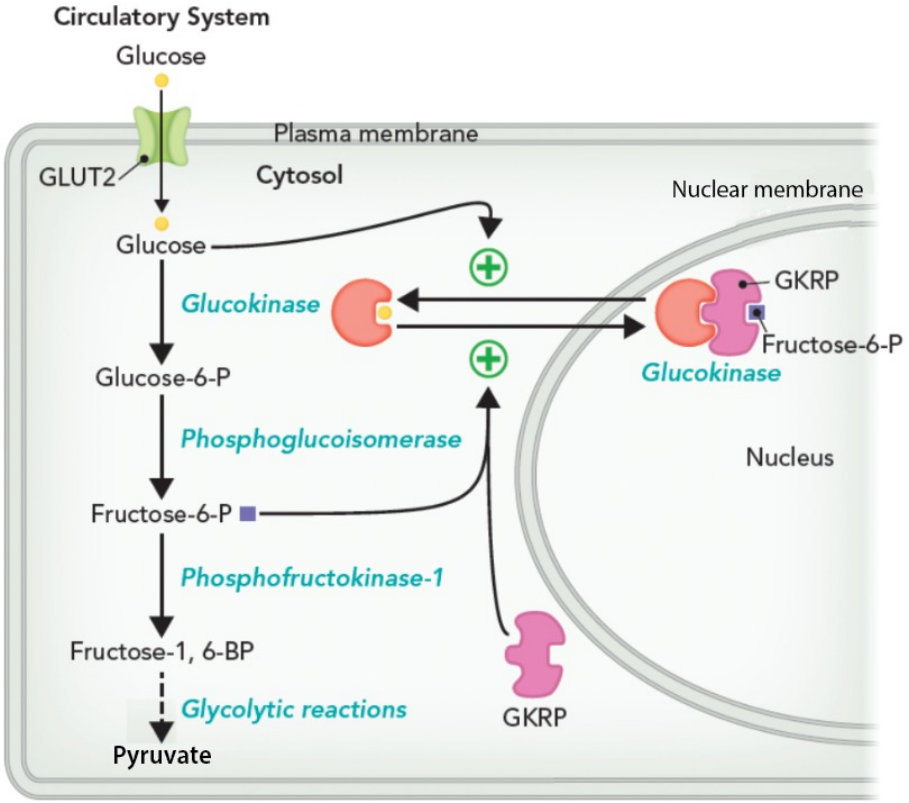
Enzyme Kinetics: Hexokinase vs. Glucokinase

- Glucokinase has a much higher K_m for glucose than hexokinase, enabling it to function as a sensor of elevated blood glucose levels in liver cells.
- Glucokinase ensures that glucose is phosphorylated and retained in liver cells only when blood glucose levels are high, promoting liver glycogen storage.



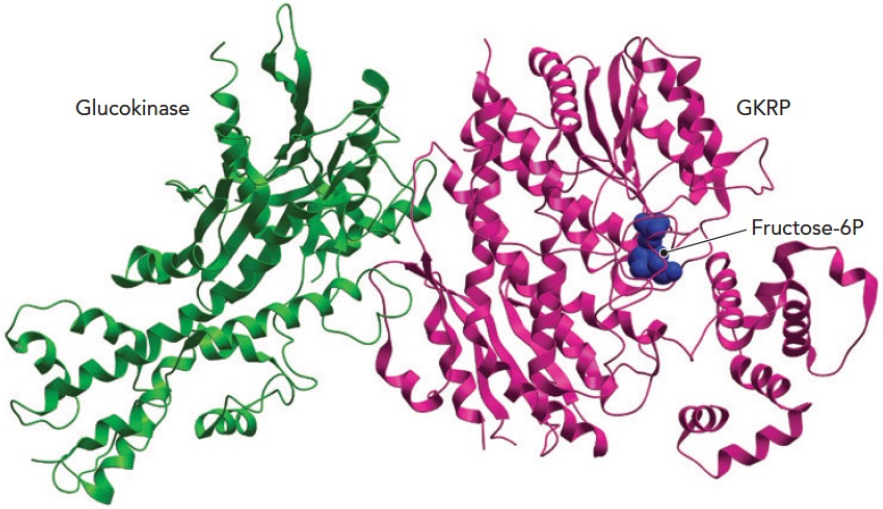
Regulation of Glucokinase by GKRP

- Glucokinase regulatory protein (GKRP) binds glucokinase in the liver under low glucose conditions and sequesters glucokinase in the nucleus; GKRP binding to glucokinase is stimulated by fructose-6P (feedback inhibition).
- When glucose levels rise, glucose displaces GKRP, allowing glucokinase to return to the cytosol and increase glycolytic flux.



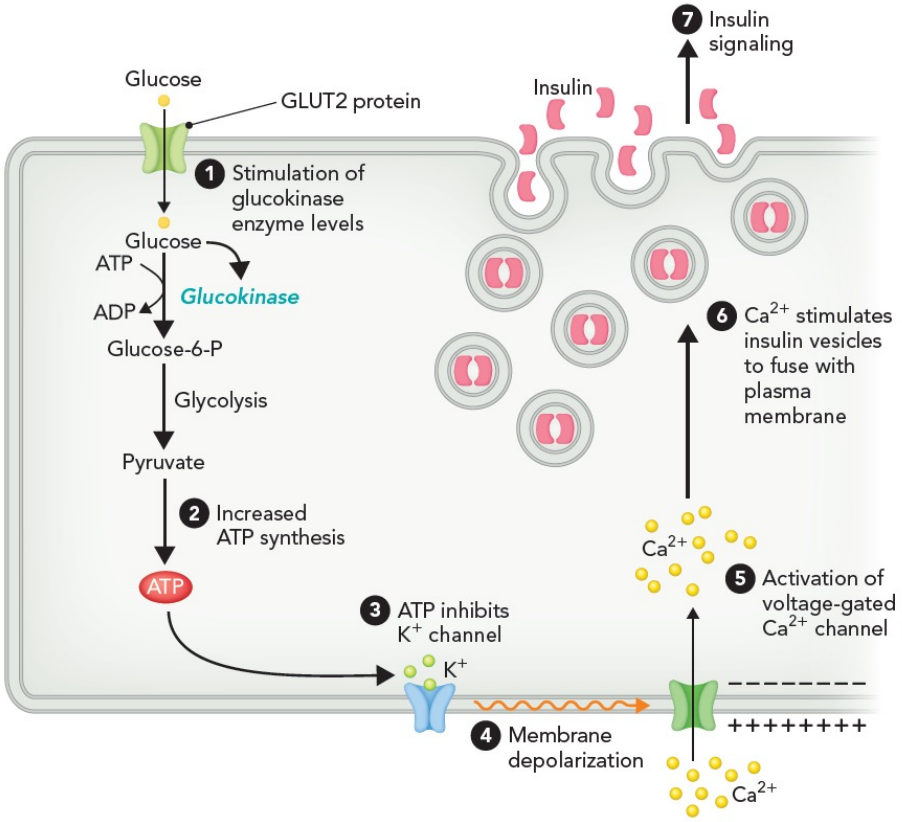
Structure of the Glucokinase-GKRP Complex

- The inhibitory complex between GKRP and glucokinase is stabilized by fructose-6-phosphate binding, facilitating nuclear sequestration.
- This structural mechanism reduces glucokinase activity when glycolytic flux needs to decrease due to high cellular energy charge (feedback inhibition by fructose-6P).



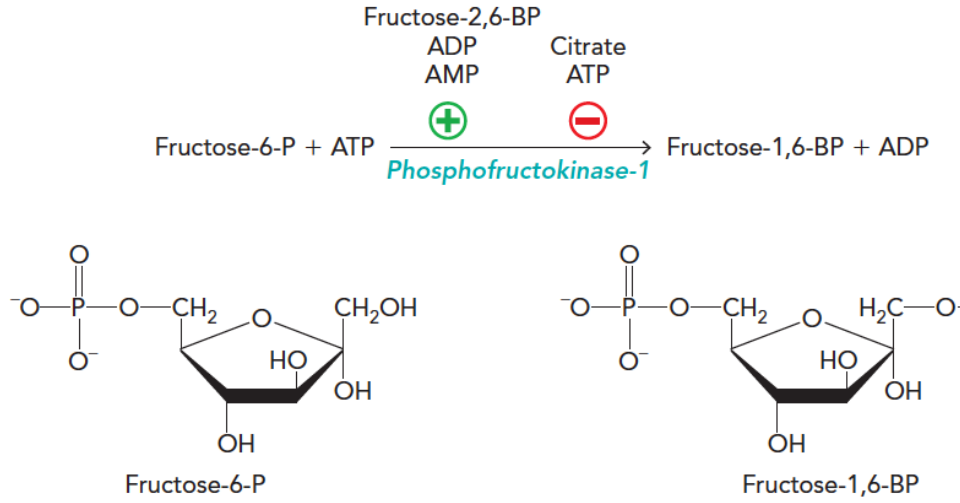
Glucokinase is a Glucose Sensor in β Cells

- Expression of glucokinase is increased in response to increased glucose import mediated by the GLUT2 transporter protein.
- Elevated flux through glycolysis results in increased ATP levels, which inhibits K^+ channels, depolarizes the membrane, opens Ca^{2+} channels, and triggers insulin release into the bloodstream to lower blood glucose levels.



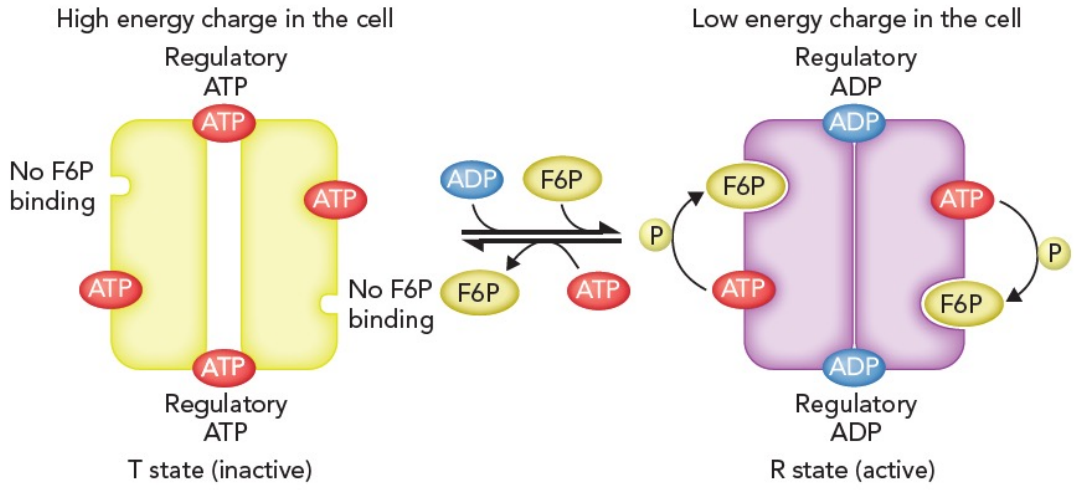
Allosteric Regulation of PFK-1 Activity

- PFK-1 is activated by AMP, ADP, and fructose-2,6-bisphosphate, which signal low energy charge and promote glycolytic flux.
- ATP and citrate inhibit PFK-1 activity, reducing glycolytic flux when energy levels are high or biosynthetic precursors accumulate.



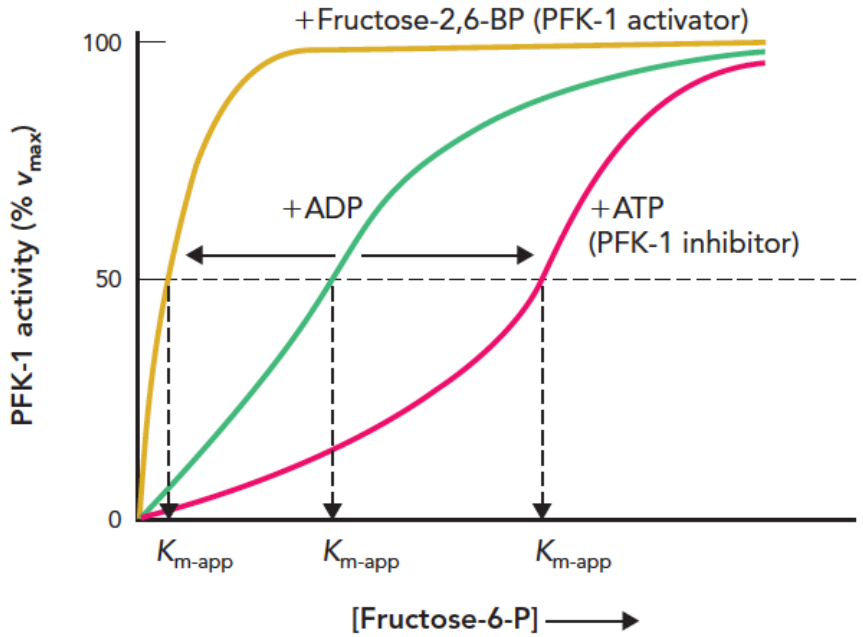
Allosteric Regulation of PFK-1 Activity

- High ATP concentrations increase levels of PFK-1 in the inactive T-state conformation, which decreases the enzyme affinity for fructose-6-P.
- High AMP and ADP concentrations increase levels of PFK-1 in the active R-state conformation, which increases to enzyme affinity for fructose-6-P.



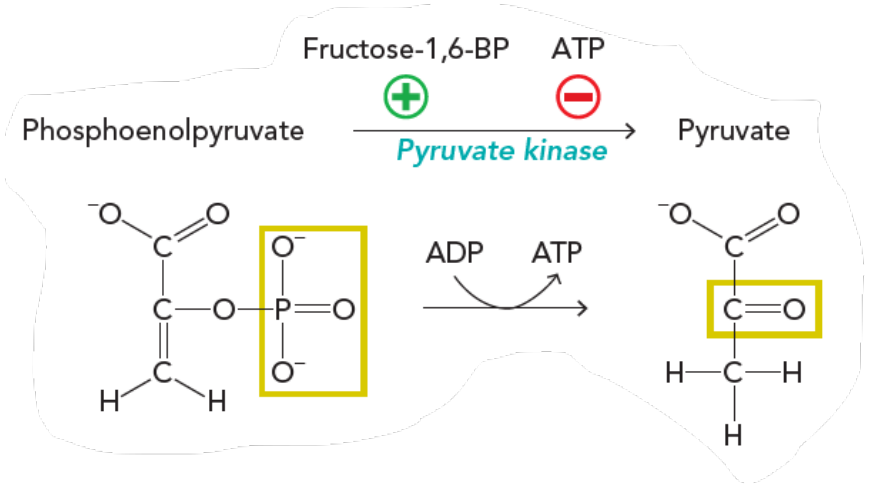
Allosteric Regulation of PFK-1 Activity

- PFK-1 activity is inhibited by high ATP concentrations, whereas PFK-1 activity is stimulated when fructose-2,6-BP levels are elevated.
- This can be seen by a decrease in the $K_{m\text{-}app}$ of PFK-1 in the presence of fructose-2,6-BP relative to the $K_{m\text{-}app}$ in the presence of ADP, in contrast, increased ATP levels result in a higher $K_{m\text{-}app}$ of PFK-1 relative to the $K_{m\text{-}app}$ in the presence of ADP.



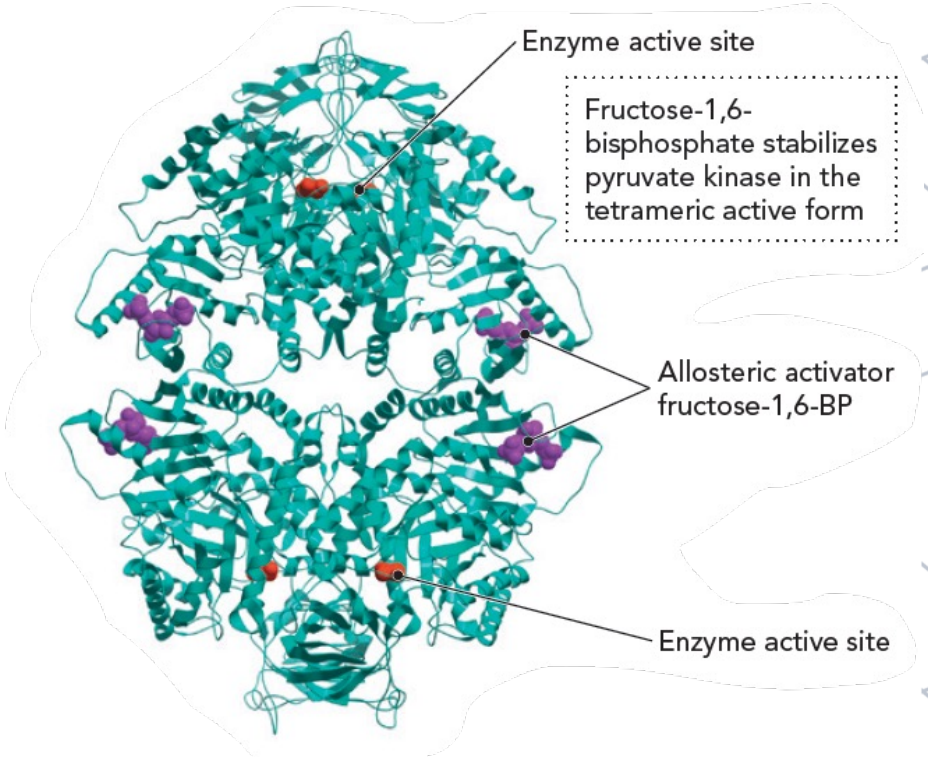
Feed Forward Activation of Pyruvate Kinase

- Pyruvate kinase is activated by fructose-1,6-bisphosphate, which signals increased upstream flux through glycolysis.
- This feed-forward regulation ensures efficient conversion of phosphoenolpyruvate to pyruvate when substrate availability is high.
- ATP inhibits the activity of pyruvate kinase, which signals high energy charge and need to decrease metabolic flux through glycolysis.



Feed Forward Activation of Pyruvate Kinase

- Fructose-1,6-bisphosphate stabilizes the active tetrameric conformation of pyruvate kinase by binding to its allosteric sites.
- This structural mechanism enhances enzyme activity to efficiently match glycolytic flux with cellular energy demands.



Key Concepts to Guide Your Learning

- Glucokinase is an isozyme of hexokinase that converts glucose to glucose-6-P in pancreatic and liver cells and has a very low affinity for glucose (100 times lower); glucokinase activity is not feedback inhibited by glucose-6-P.
- Liver glucokinase is regulated by GKRP, which sequesters glucokinase in the nucleus; when glucose levels are high, glucose outcompetes GKRP for binding to to glucokinase and facilitates glucokinase export back to the cytoplasm.
- Phosphofructokinase-1 (PFK-1) is allosterically regulated by energy charge and by flux through the glycolytic and citrate cycle pathways; PFK-1 is activated by AMP, ADP, and fructose-2,6-bisphosphate and is inhibited by ATP and citrate.
- Pyruvate kinase is activated by fructose-1,6-bisphosphate, which binds to and stabilizes the active tetrameric form of the enzyme (feed-forward regulation); ATP (high energy charge) is an allosteric inhibitor of pyruvate kinase activity.