BioChem 330 - Course Outline

October 25-Nov 3, 2011

- **Bio-molecular Structure/Function (I cont'd)**
  - NUCLEIC ACID
    - DNA sequence and structure
    - Protein/nucleic acid interactions
  - CARBOHYDRATES
    - Sugars - mono and disaccharides
    - Polysaccharides
    - Glycerides and glycerol
  - FATS AND LIPIDS
    - Chemistry and nomenclature for fatty acids
    - Saturated and unsaturated fatty acids
    - Fluid mosaic model of membrane structure

BioChem 330 - Course Outline

- **Metabolism and Bioenergetics (II)**
  - ENZYME CATALYSIS:
    - kinetic constants k_{cat}, K_m
    - Catalytic strategies, the serine proteases
  - CATABOLISM (*breakdown*)
    - Carbohydrates
      - Glycolysis
      - Tricarboxylic Acid Cycle
      - Electron Transport
      - Chemiosmosis and ATPase
    - Fatty acids and amino acids

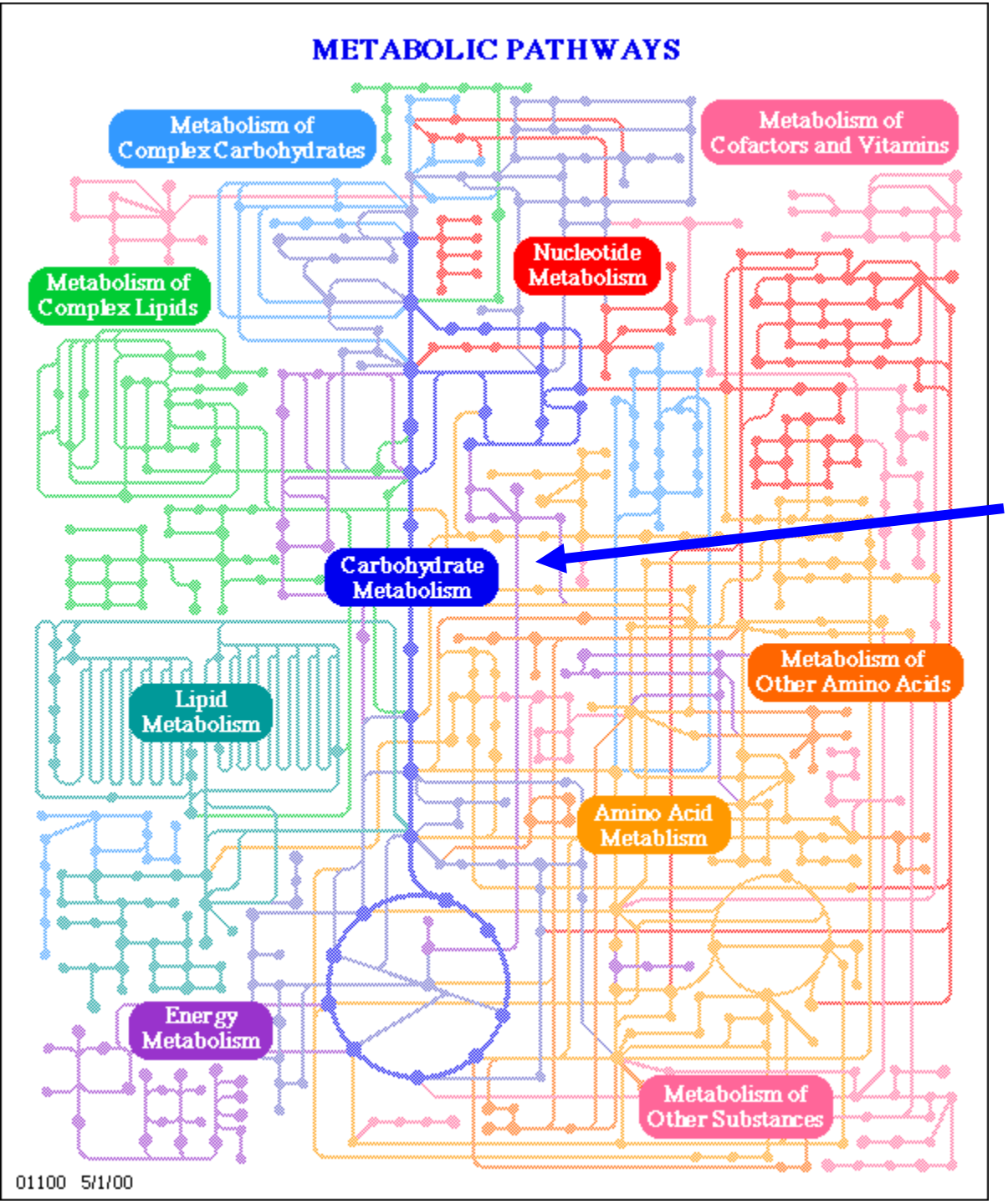
Carbohydrate Metabolism

October 25-Nov 3, 2011

- Intro to Metabolism
  - –ATP, the energy currency of the cell
  - –sugar structure
- Glycolysis Phase I
  - – gly 1-5
- Glycolysis Phase II
  - – gly 6-10
- Control in Glycolysis

Metabolism

- Catabolism
  - Degradation reactions
  - More complex to simpler molecules
- Anabolism
  - Biosynthesis
  - Less complex to more complex molecules
- Entropy:
  - Catabolism is favorable, and should have $\Delta G^{o'} < 0$
  - Anabolism is unfavorable, and should have $\Delta G^{o'} > 0$



Over 1000 different reactions are being catalyzed in an *e.coli* cell, we'll focus on about two dozen of them which form the blue central line of carbohydrate metabolism.

http://www.genome.jp/kegg/

Why must a living organism metabolize nutrients such as fats, proteins, carbohydrates?

ANS: To generate ATP, the fuel that drives all cellular events

- $ATP^{-4} + H_2O \longrightarrow ADP^{-3} + PO_4^{-3} + 2H^+$ $\Delta G^{o'} = -30.5$ kJ/mole

ATP hydrolysis liberates energy making it available for biological processes that require energy

Phosphate Hydrolysis Potential Energy

- Molecules higher in table can transfer a phosphate group to other molecules below them in table (so net $\Delta G^{o'} < 0$)
- A reaction far away from its equilibrium ratio of prod/react and from the standard state can have additional free energy.
- In the case of ATP hydrolysis, cells can be many orders of magnitude away from equilibrium and are certainly NOT at the standard state. How does this affect the free energy available from hydrolysis?

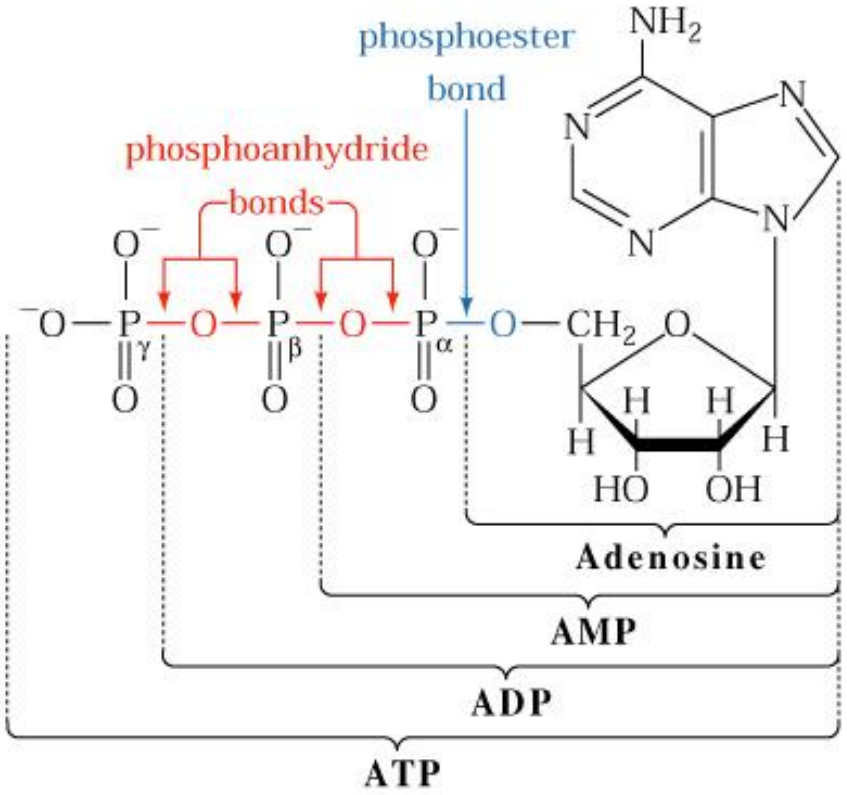
Table 13-2. **Standard Free Energies of Phosphate Hydrolysis of Some Compounds of Biological Interest**

Compound	$\Delta G^{o'}$ $(kJ \cdot mol^{-1})$
Phosphoenolpyruvate	−61.9
1,3-Bisphosphoglycerate	−49.4
Acetyl phosphate	−43.1
Phosphocreatine	−43.1
PP_i	−33.5
ATP (→ AMP + PP_i)	−32.2
ATP (→ ADP + P_i)	−30.5
Glucose-1-phosphate	−20.9
Fructose-6-phosphate	−13.8
Glucose-6-phosphate	−13.8
Glycerol-3-phosphate	−9.2

Source: Jencks, W.P., in Fasman, G.D. (Ed.), *Handbook of Biochemistry and Molecular Biology* (3rd ed.), Physical and Chemical Data, Vol. 1, pp. 296–304, CRC Press (1976).

Which phosphate bonds in ATP and ADP are “high-energy” bonds?

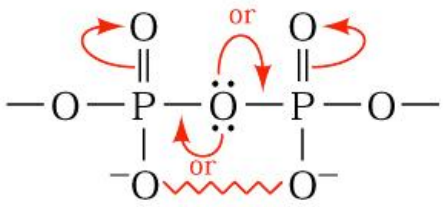
- Breakdown of O-P phosphoan-hydride bond at P_γ of ATP produces ADP and P_i and energy.
- Breakdown of O-P phosphoan-hydride bond at P_β of ADP produces AMP and P_i and energy.
- Breakdown of O-P phosphoan-hydride bond at P_β from ATP produced PP_i and AMP and energy and then some.



Why is the phosphoanhydride bond a "high-energy" bond?

- LOTS of energy is released due to
  - destabilization of reactants (electrostatic repulsion due to multiple negative charges)
  - stabilization of products (two resonance forms rather than 1 plus products are more easily hydrated b/c charge is more spread out)

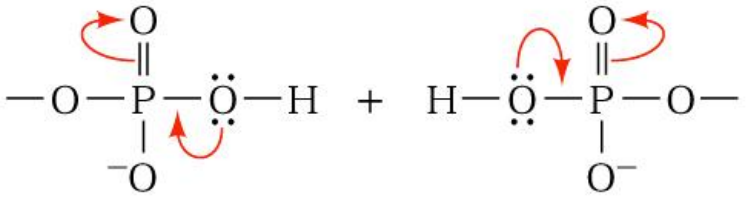
Destabilized by electrostatic repulsion of negative charges at O^-



:OH_2 attacks at $P\gamma$ or P_β

H_2O

better resonance stabilization



Energetics of *making* ATP

- **ADP + PO_4^{-3} + H^+ = ATP + H_2O** $\Delta G^{o'}$ = +30.5 kJ/mole

It takes 30.5 kJ to make one mole of ATP under biological standard state conditions (1 M reactants and products and pH 7.0 295 K) . At equilibrium…….

$K' = \exp(-\Delta G^{o'}/RT)$

$= 5 \times 10^{-6}\ M^{-1}$

= [Prod] / [Reactants]

$= [ATP] / [ADP][P_i]$

= 1/200,000

But how far are cells away from equilibrium with regard to the synthesis of ATP?

Energetics of making ATP *under cellular conditions*

- **ADP + PO_4^{-3} + H^+ ≠ ATP + H_2O** $\Delta G = ?$

•Cells are far away from equilibrium and far away from standard state conditions. We have **much** more ATP than would be dictated by equilibrium; the ratio of ATP to ADP+P_i in some cells is as high as 200/1 rather than 1/200,000.

•This means that a cell can be far from equilibrium w.r. to this ratio, and now, through metabolism, we are going to make EVEN MORE ATP.

•Under these conditions, thermodynamics wants the system instead to hydrolyze the ATP and reach equilibrium, but instead, metabolic reactions will need to fight equilibrium; let's calculate exactly how much more energy it will take to make this ATP:

Energetics of making ATP *under cellular conditions*

- **ADP + PO_4^{-3} + H^+ ⇌ ATP + H_2O** $\Delta G = ?$

To calculate this energy, remember from thermodynamics:

$$\Delta G = \Delta G^{o'} + RT \ln Q^*$$

*(*At equilibrium, this equation simplifies to the more familiar eqn. $\Delta G^{o'} = - RT \ln K$ because $\Delta G = 0$)*

Where ΔG is the free energy available to the system ***not at equilibrium***, and Q is the mass action ratio:

Q = [Products]/[Reactants] when a cell is away from equilibrium….

so what is this value in an average cell?

Energetics of making ATP *under cellular conditions*

- **ADP + PO_4^{-3} + H^+ ⇌ ATP + H_2O** $\Delta G = ?$

In human erythrocytes for example:

$Q = [ATP] / [ADP][P_i]$

$= 2.25 \times 10^{-3} / (0.25 \times 10^{-3} \times 1.65 \times 10^{-3)} = 5.45 \times 10^3\ M^{-1}$

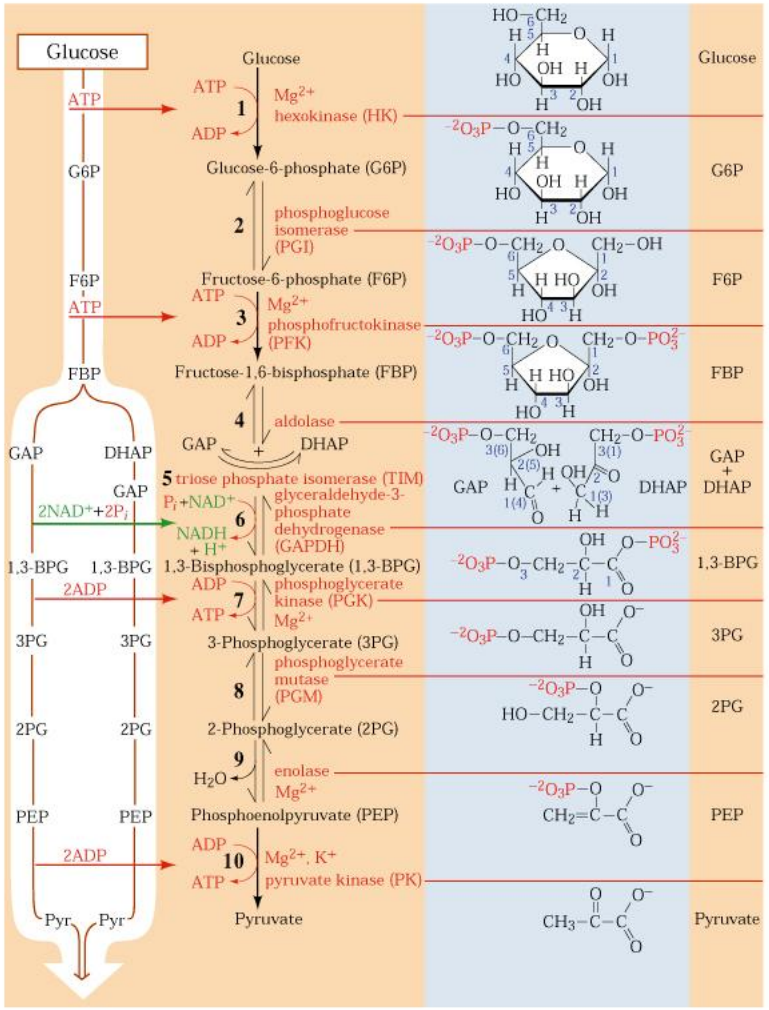
$\Delta G = \Delta G^{o'} + RT \ln Q$

$= 30.5$ kJ/mole + 21 kJ/mole

$= +51.5$ kJ/mole

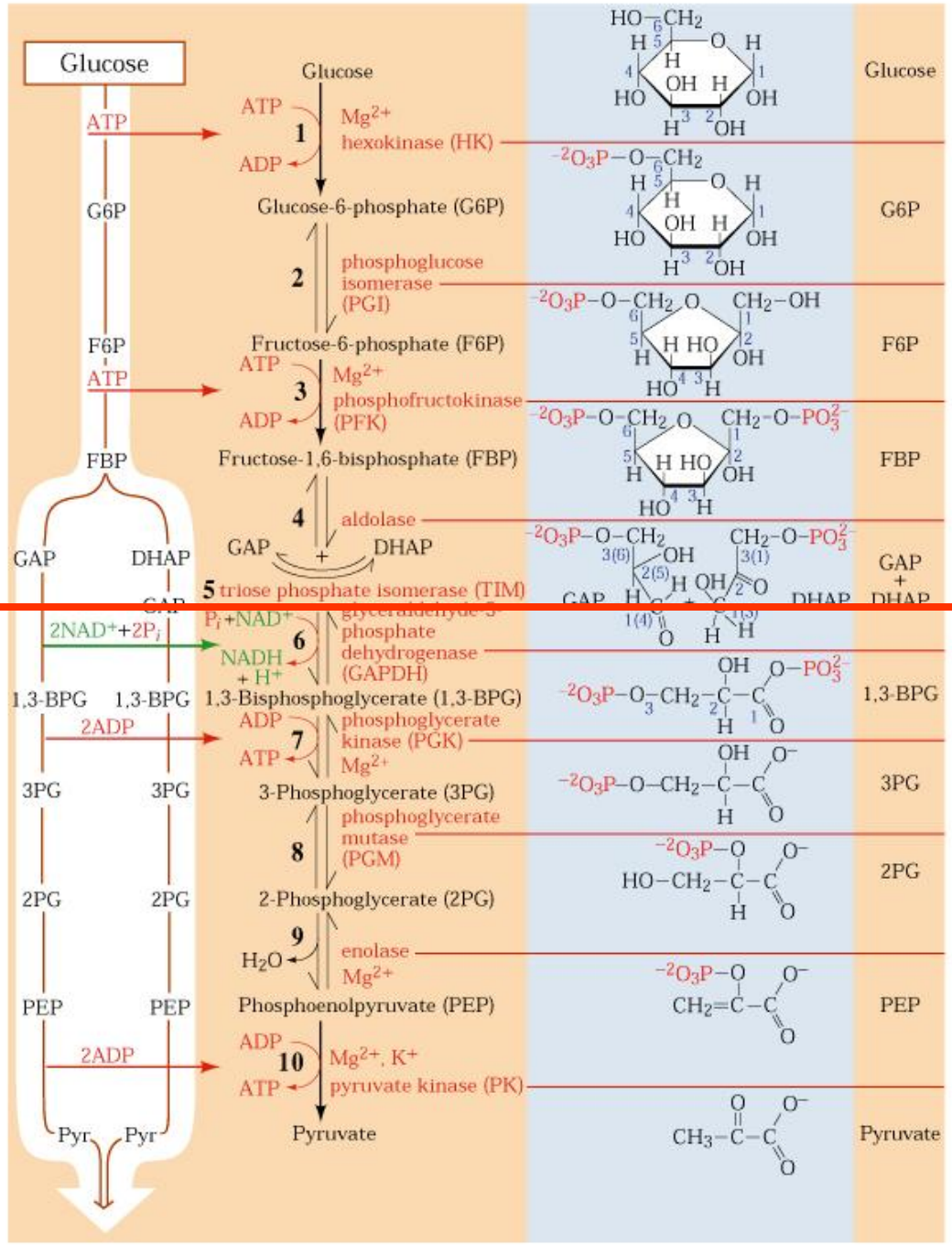
Overview Of Glycolysis

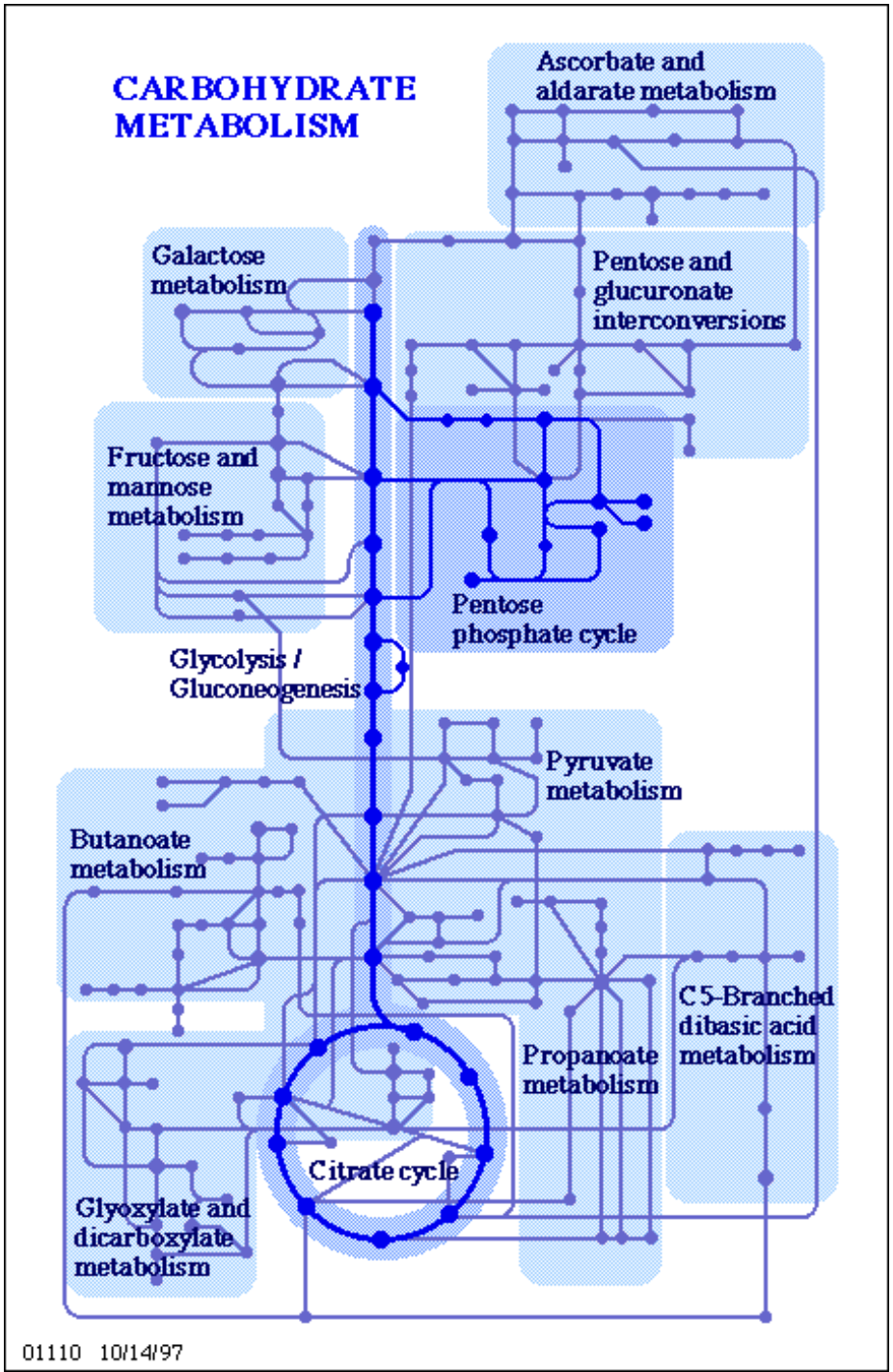
- Stage I
  - Uses 2 ATP's per glucose
- Stage II
  - Generates 4 ATP's



PHASE I

PHASE II




CARBOHYDRATE METABOLISM
Ascorbate and aldarate metabolism
Galactose metabolism
Pentose and glucuronate interconversions
Fructose and mannose metabolism
Pentose phosphate cycle
Glycolysis / Gluconeogenesis
Pyruvate metabolism
Butanoate metabolism
C5-Branched dibasic acid metabolism
Propanoate metabolism
Citrate cycle
Glyoxylate and dicarboxylate metabolism
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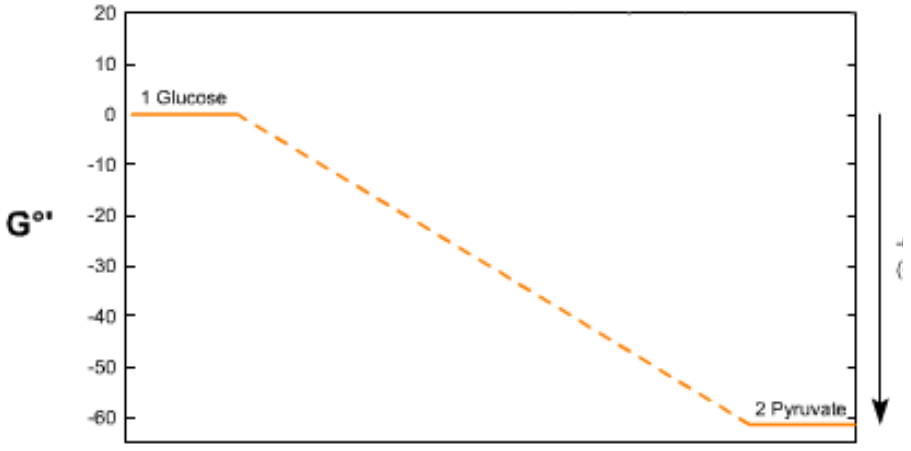
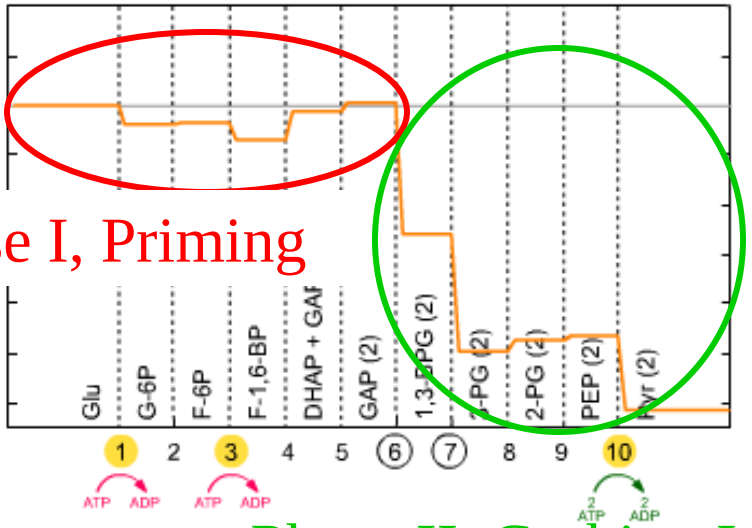
Glycolysis: **a biochemical analogue of the movie "Roots"**

Glucose+2P_i+2ADP+2NAD^+ = 2pyruvate+2ATP+2NADH+2H^++2H_2O

$\Delta G^{o'}$ = **-85** kJ/mole

(Note above NET reaction of glycolysis book keeps important biological molecules but is not charge balanced)





Phase I, Priming

Phase II, Cashing In

Phase I

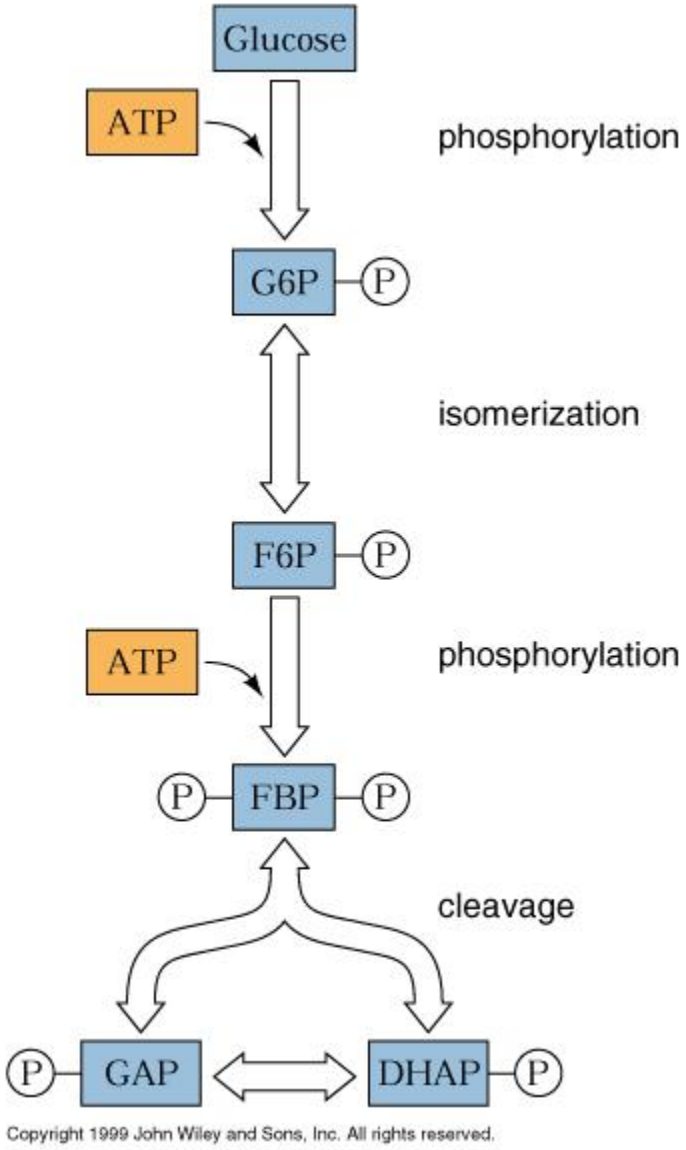
Glucose + 2 ATP = 2 GAP + 2 ADP

Priming the Pump in five steps

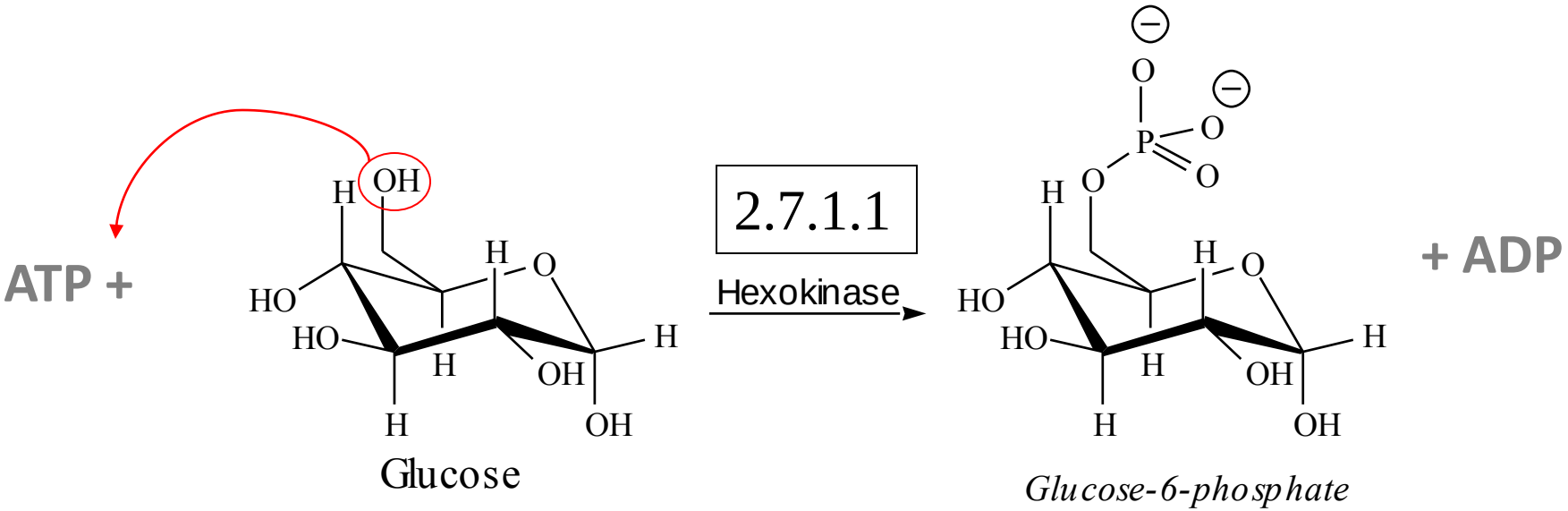
* This phase of glycolysis requires the investment of two molecules of ATP.

* Gly3, PFK, is the committed step for glycolysis.

* No oxidation and no involvement of molecular oxygen.



Gly 1 hexokinase: first ATP utilization



* ATP is the second substrate, ADP the second product.

* Reaction type is phosphoryl shift: hexokinase catalyzes the transfer of the g phosphate group from ATP to glucose, C6 OH acting as a nucleophile attacks the P of terminal phosphate.

What about the sugar cyclization?

What about the transition state?

What about metals?

$\Delta G^{o'}$ = -16.7 kJ/mole

Gly 1 hexokinase, glucokinase:

* Hexokinase shows multisubstrate binding, product inhibition, and induced fit.

* $K_{m\ glucose}$ is 0.1 mM, in erythrocytes [glucose] = 5 mM

  K_m/S is <<< 1, enzyme is saturated with glucose and operating at V_{max}

* $K_{m\ ATP}$ is 4.0 mM in the absence of glucose --- weak binding

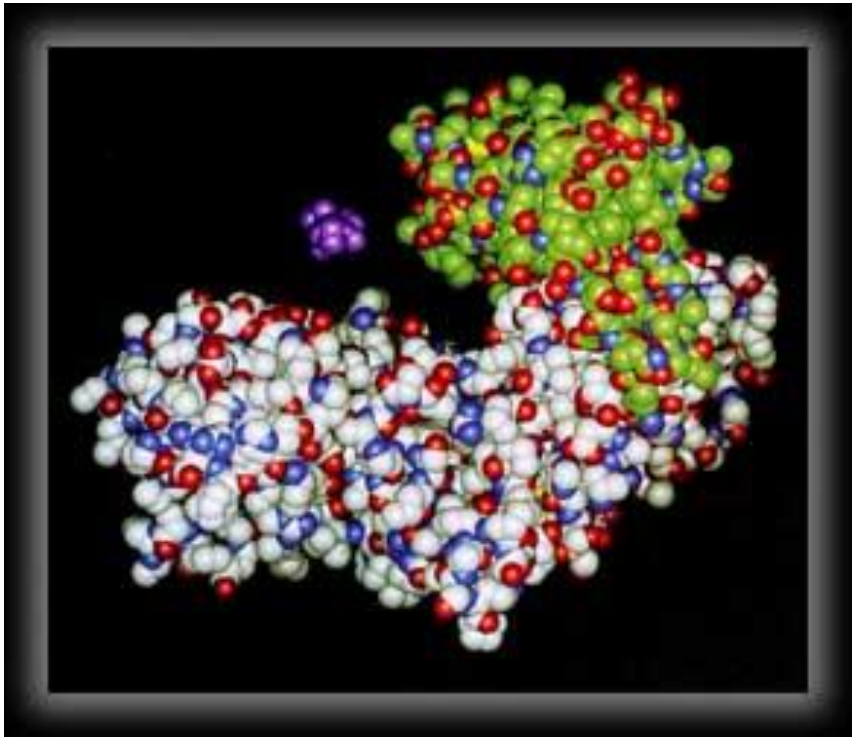
  $K_{m\ ATP}$ is 0.1 mM in the presence of glucose --- tighter binding

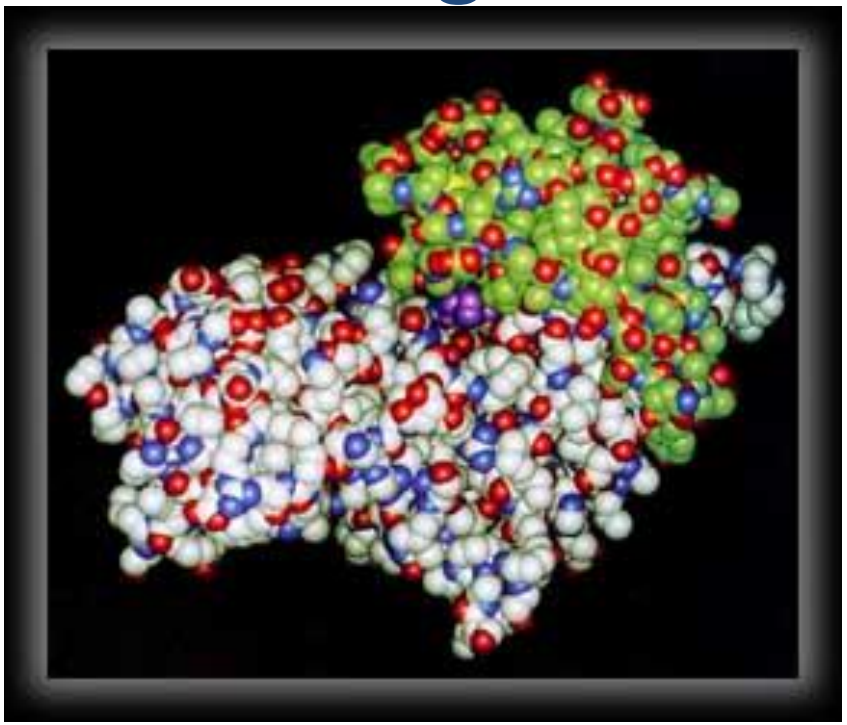
glucose binding increases affinity of enzyme 40 fold for ATP

In liver, glucokinase is an isozyme of hexokinase, only binds glucose, only activates at higher glucose levels and doesn't show product inhibition, precedes storage of glucose as glycogen, a process unique to liver and muscle cells.

All K_m, S values throughout this handout are taken from Enzyme Structure and Mechanism by Alan Fersht, p256

Gly 1 hexokinase: Induced Fit, Hinge Motion upon Binding



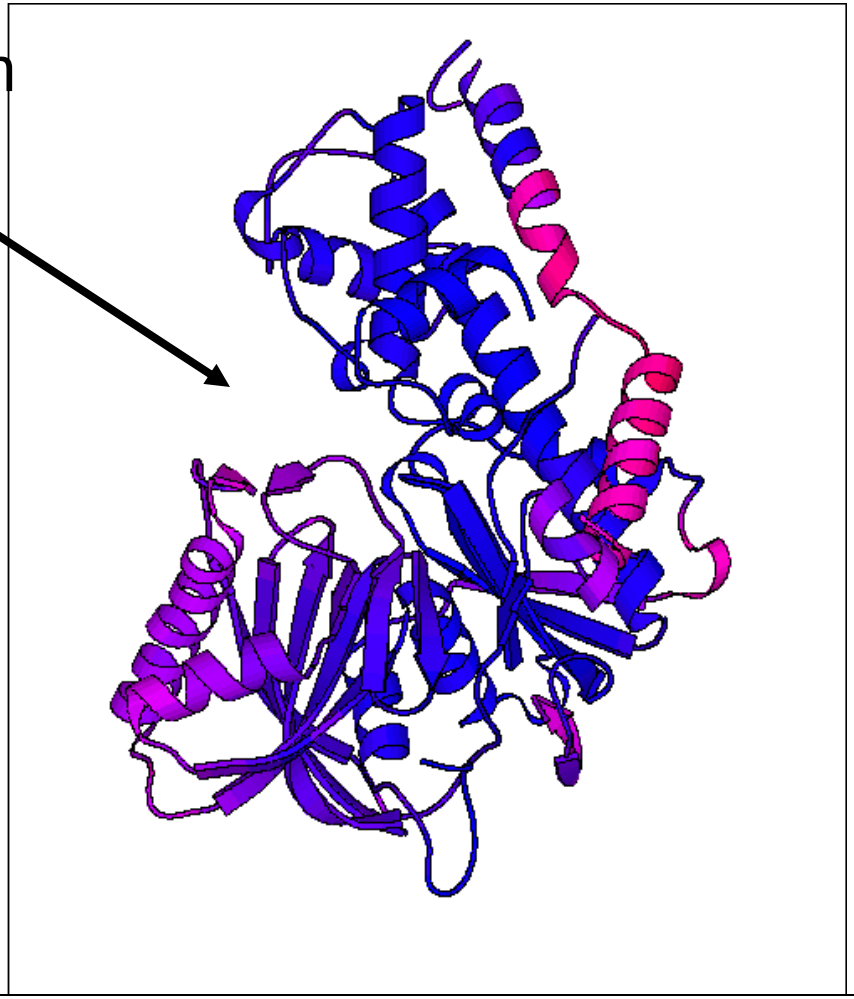


Example of Induced Fit: Protein folds over substrates to exclude water, and catalyze reaction by proximity and orientation. Two lobes rotate by 12^{o} resulting in relative movements of 8Å .

Ligands that bind but do not induce conformational change are not substrates. Product dissociation is quite slow and rate limiting.

Gly 1 yeast hexokinase, structure

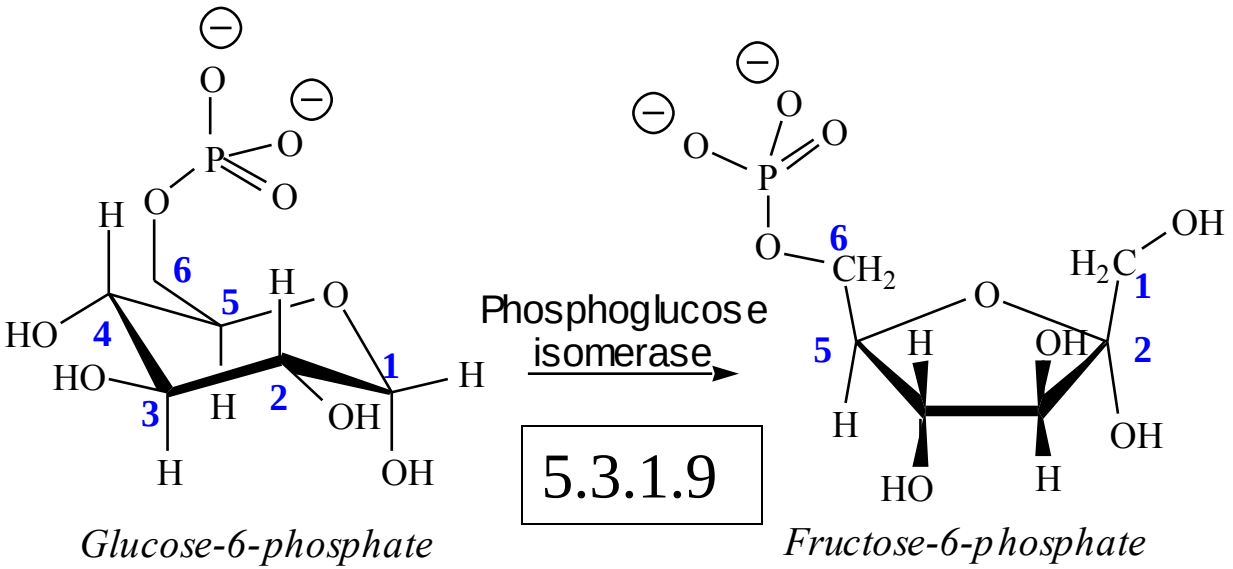
Substrates bind at domain interface



Hinge motion of protein domains: from Gerstein's Lab at Yale
http://bioinfo.mbb.yale.edu/MolMovDB/
Steitz, Shoham, Bennett Jr.: Structural dynamics of yeast hexokinase during catalysis. Philos Trans R Soc Lond B Biol Sci 293 pp. 43 (1981)

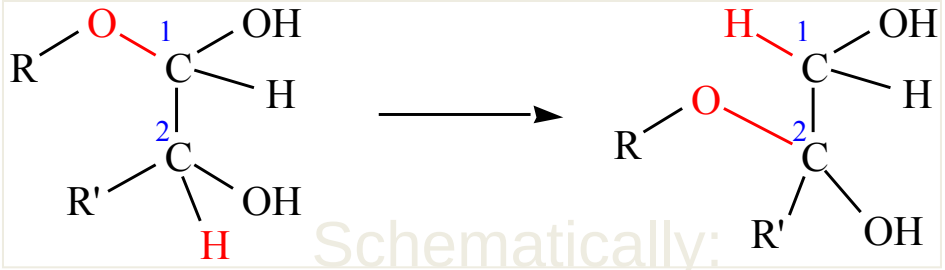
Image of hexokinase, PDB coordinates 2e2n.pdb vs 2e2q.pdb are morphed to represent the motion in the protein upon binding.

Gly 2 phosphoglucose isomerase



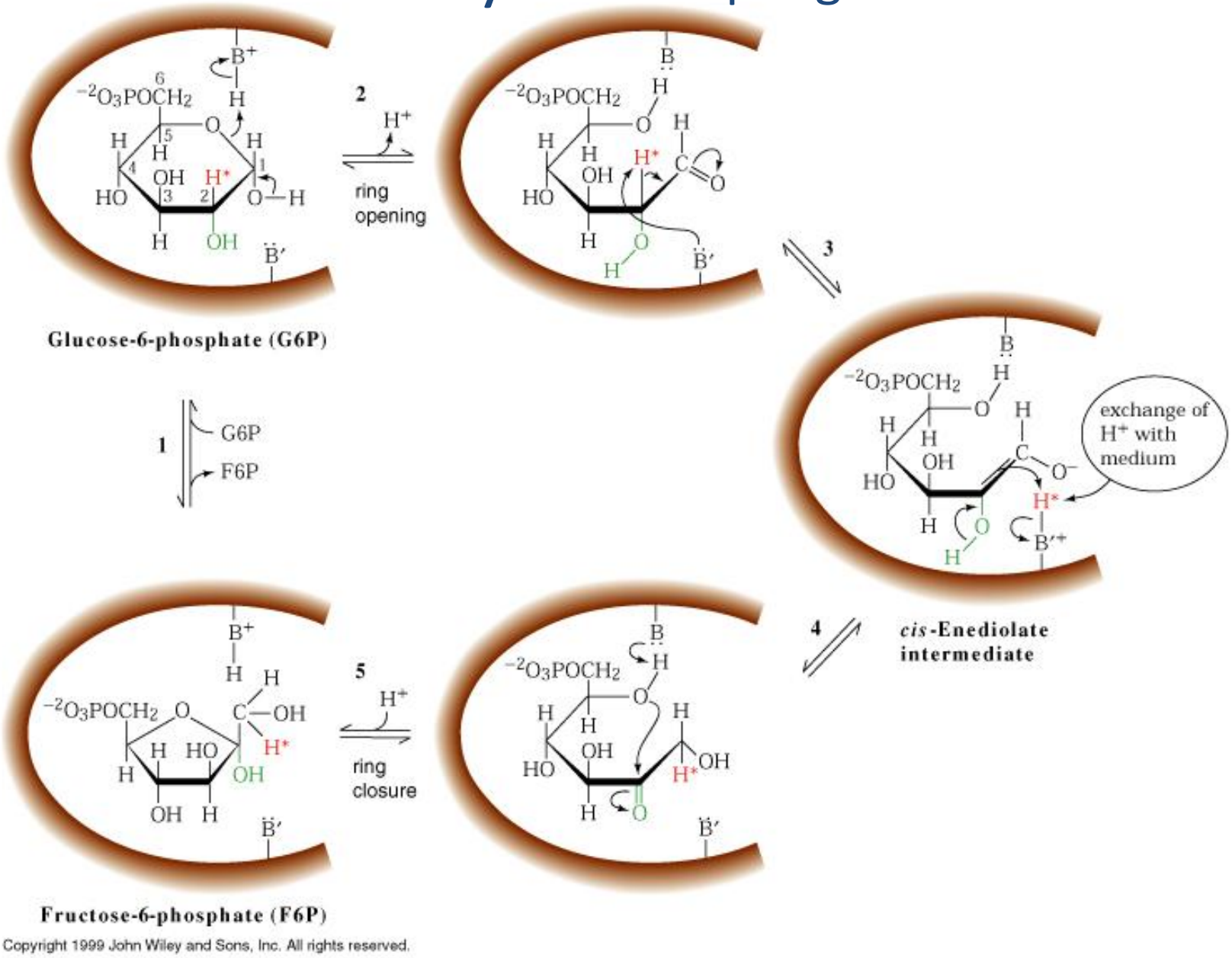
* Rxn type is isomerization: gly2 catalyzes the conversion of aldo sugar to keto sugar.

* ΔG′ about zero, reaction is almost at equilibrium

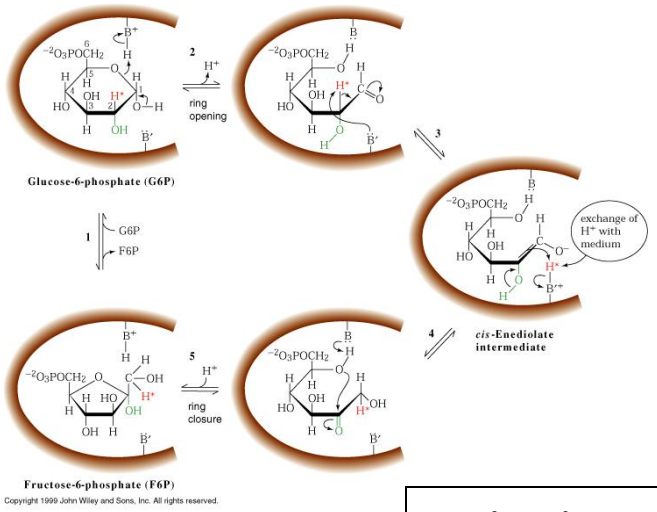


$\Delta G^{o'}$ = +1.7 kJ/mole

Mechanism of Gly 2: Phosphoglucose Isomerase



Mechanism of Gly 2: Phosphoglucose Isomerase



What would the free energy profile for this reaction look like?

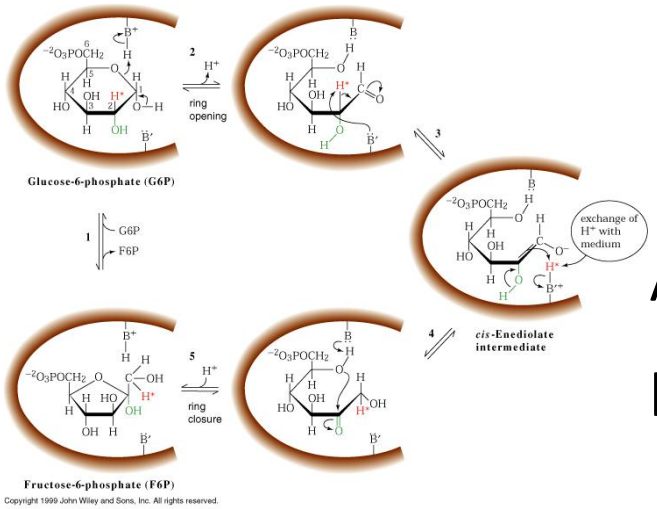
Assume: K_m = 100 uM

k_{cat} = product dissociation = $10^3 s^{-1}$

Activation Barrier = ΔG^{tt} = [17.6 - 1.36 log k_{cat}]

Binding Energy= ΔG^o = = - RTln(1/Km) = - 5 kcal

Mechanism of Gly 2: Phosphoglucose Isomerase

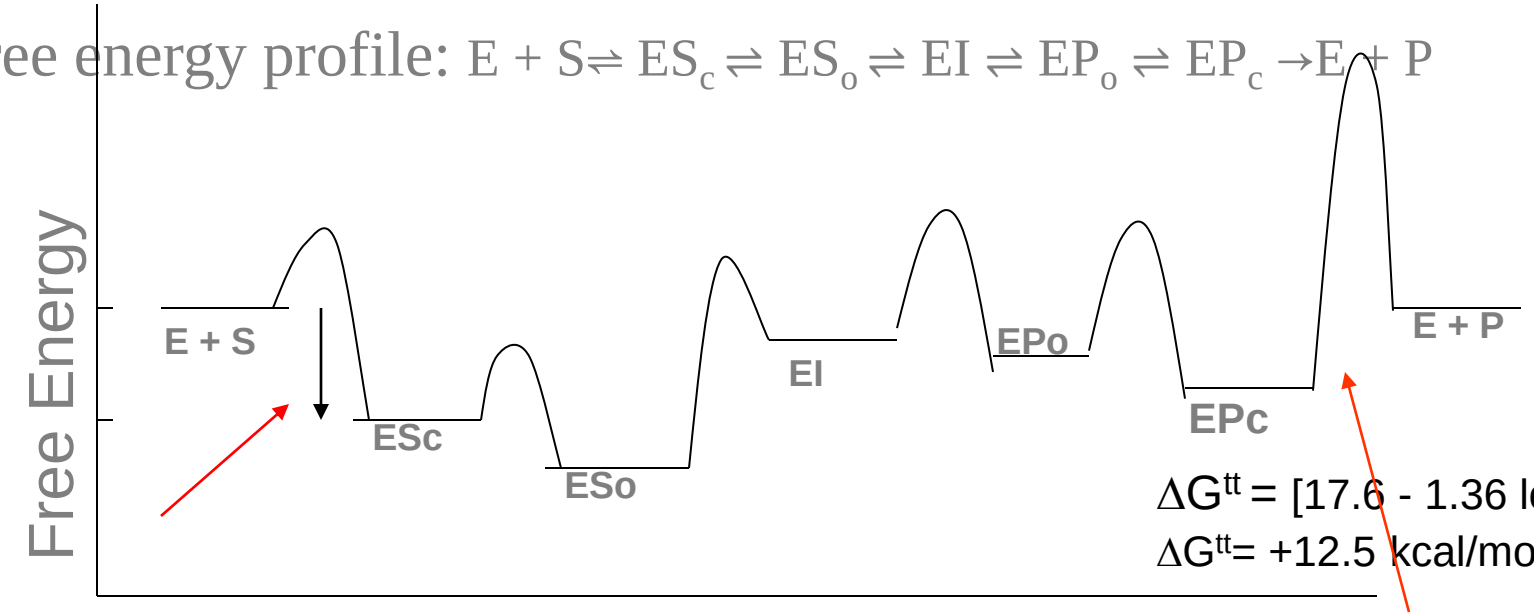


What would the free energy profile for this reaction look like?

Assume: K_m = 100 uM

k_{cat} = product dissociation = $10^3 s^{-1}$

Free energy profile: $E + S \rightleftharpoons ES_c \rightleftharpoons ES_o \rightleftharpoons EI \rightleftharpoons EP_o \rightleftharpoons EP_c \rightarrow E + P$



$\Delta G^{tt} = [17.6 - 1.36 \log k_{cat}]$

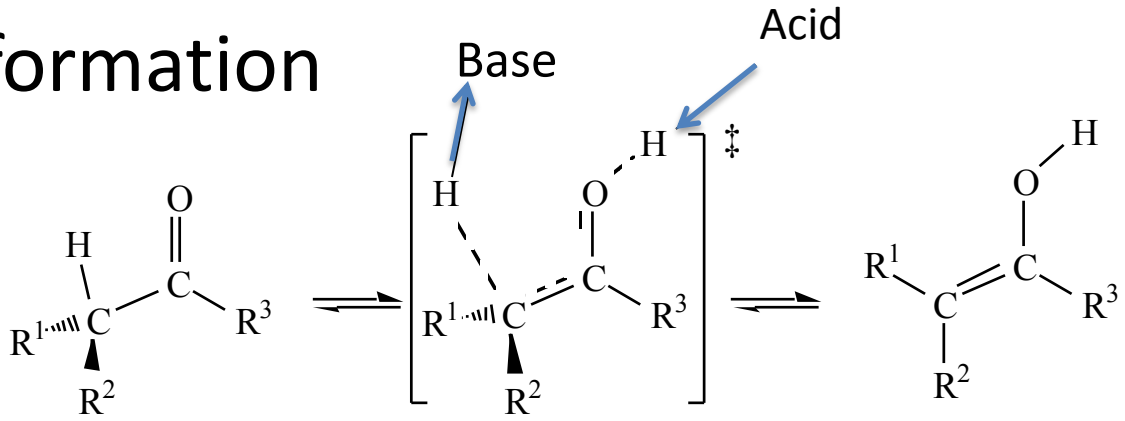
ΔG^{tt}= +12.5 kcal/mole

ΔG^{tt} =+ 50.0 kJ/mole

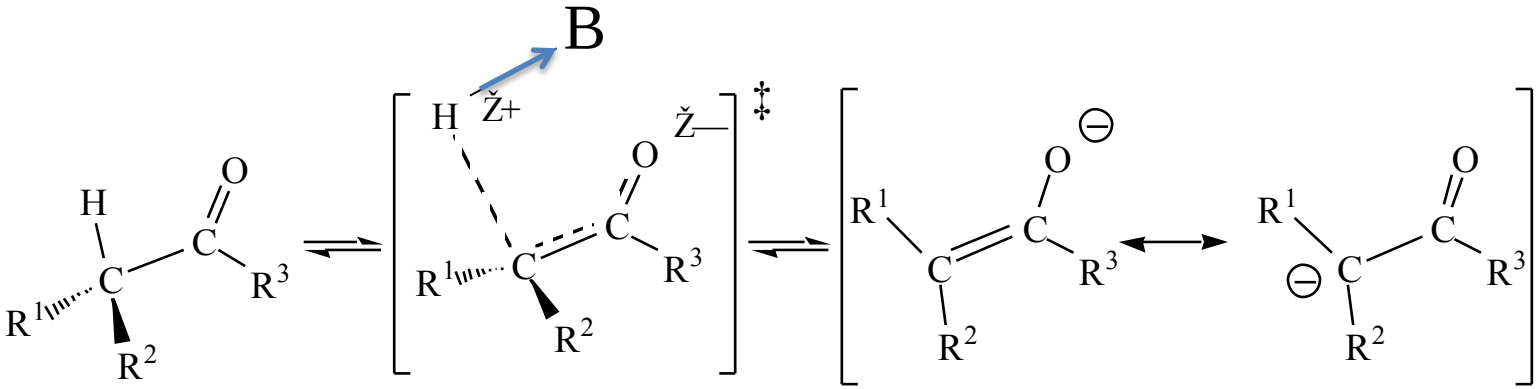
ΔG = Binding Energy= - RTln(1/Km) = - 5 kcal

Gly 2 Mechanism: Enolate formation

- Enol formation

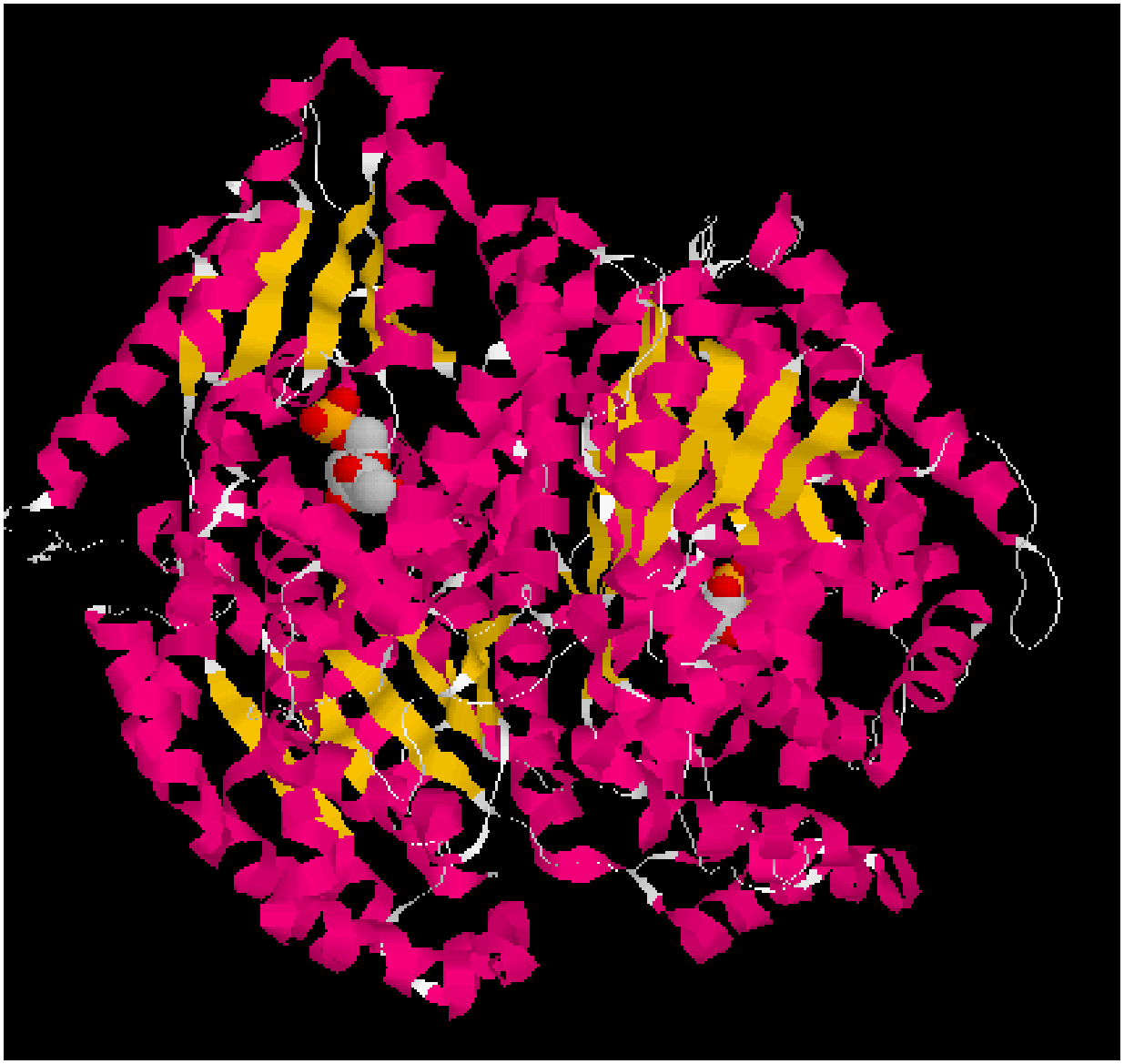


- Enolate formation



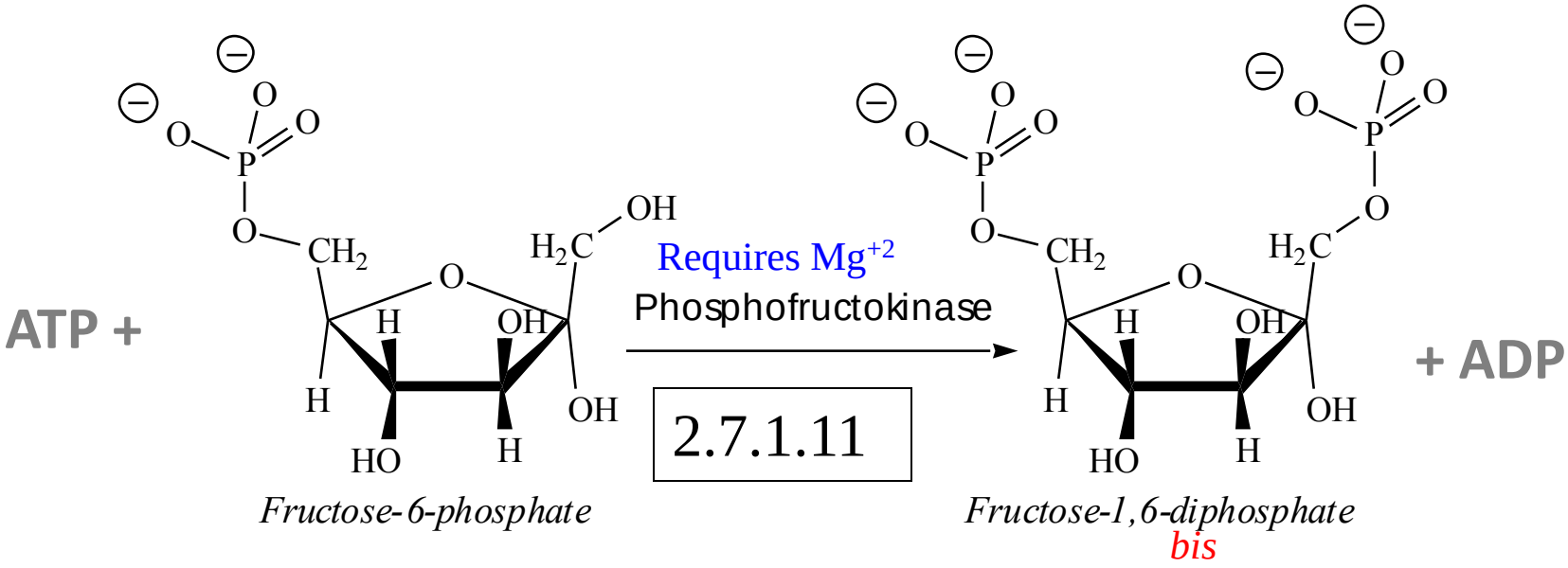
Gly 2 Structure 1 HOX from Rabbit

2.0 Å resolution structure of a dimer from rabbit phosphogluco-isomerase with 2 f6p, one bound in center of each monomer.



Gly 3 Phosphofructokinase

Second ATP Utilization



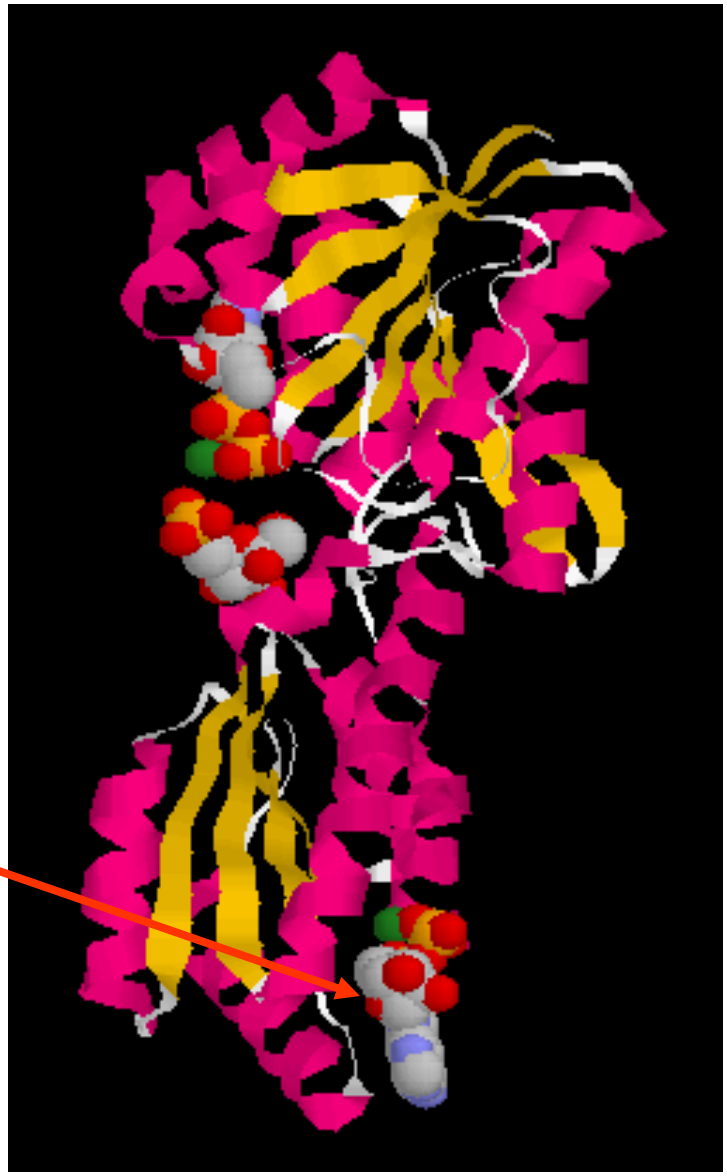
Committed (irreversible) step of glycolysis

$\Delta G^{o'}$ = -14.2 kJ/mole

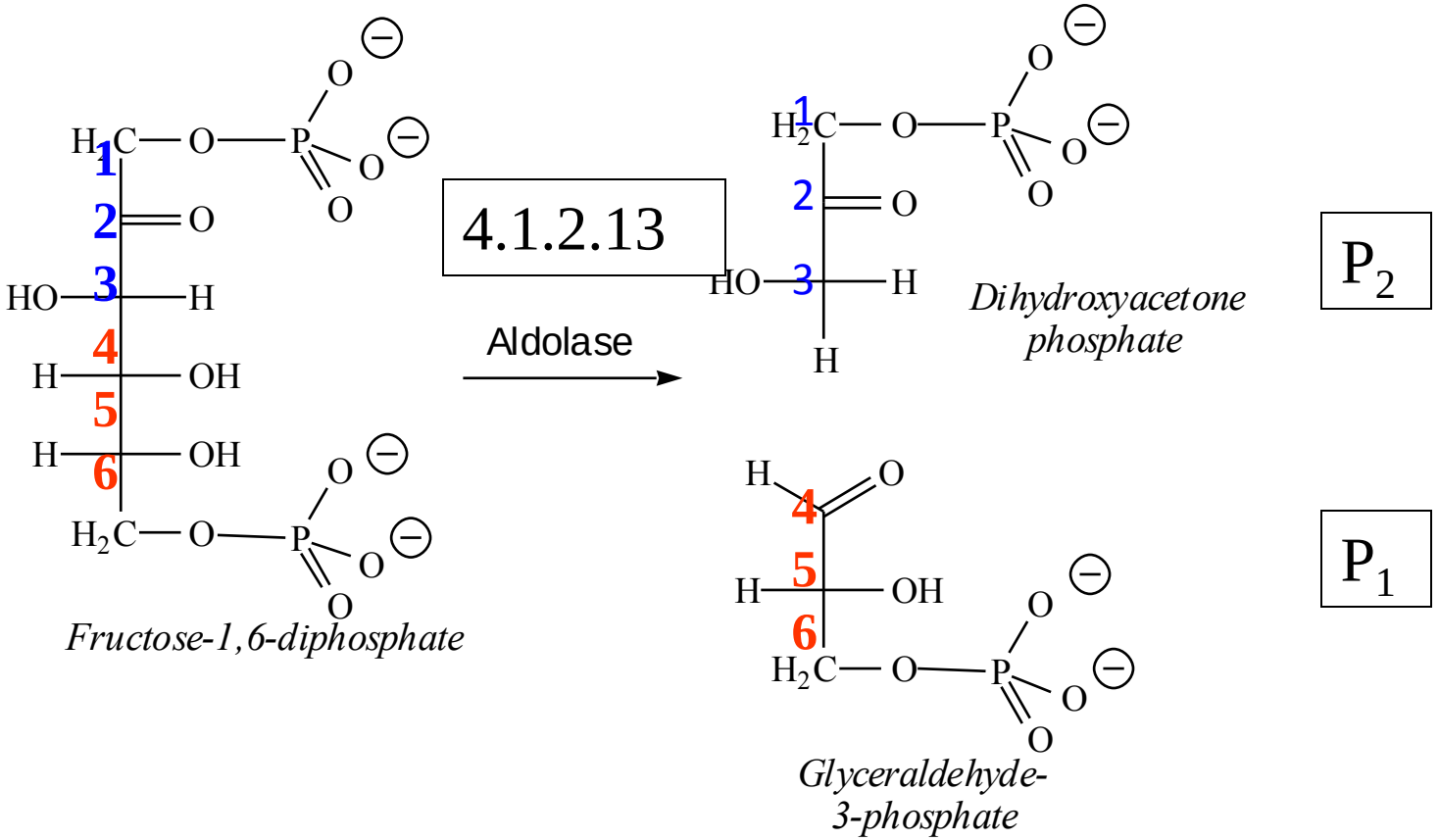
highly regulated: + regulators: Pi, AMP, - regulators: ATP, fatty acids, citrate, H+ also **de**inhibitors of ATP inhibition: F2-6BP (later)

Gly 3: Structure of 1PFK from *e. coli*

(PFK1)Glycolysis:"Ribbon diagram of Phospho- Fructokinase, Gly-3. PFK catalyzes the conversion of F6P to F(1,6)BP. Here, the substrate F6P is shown bound at the center. A Mg(II) ion and ADP product molecule are adjacent to F6P. At the other end of the molecule, ADP is bound at a regulatory site."



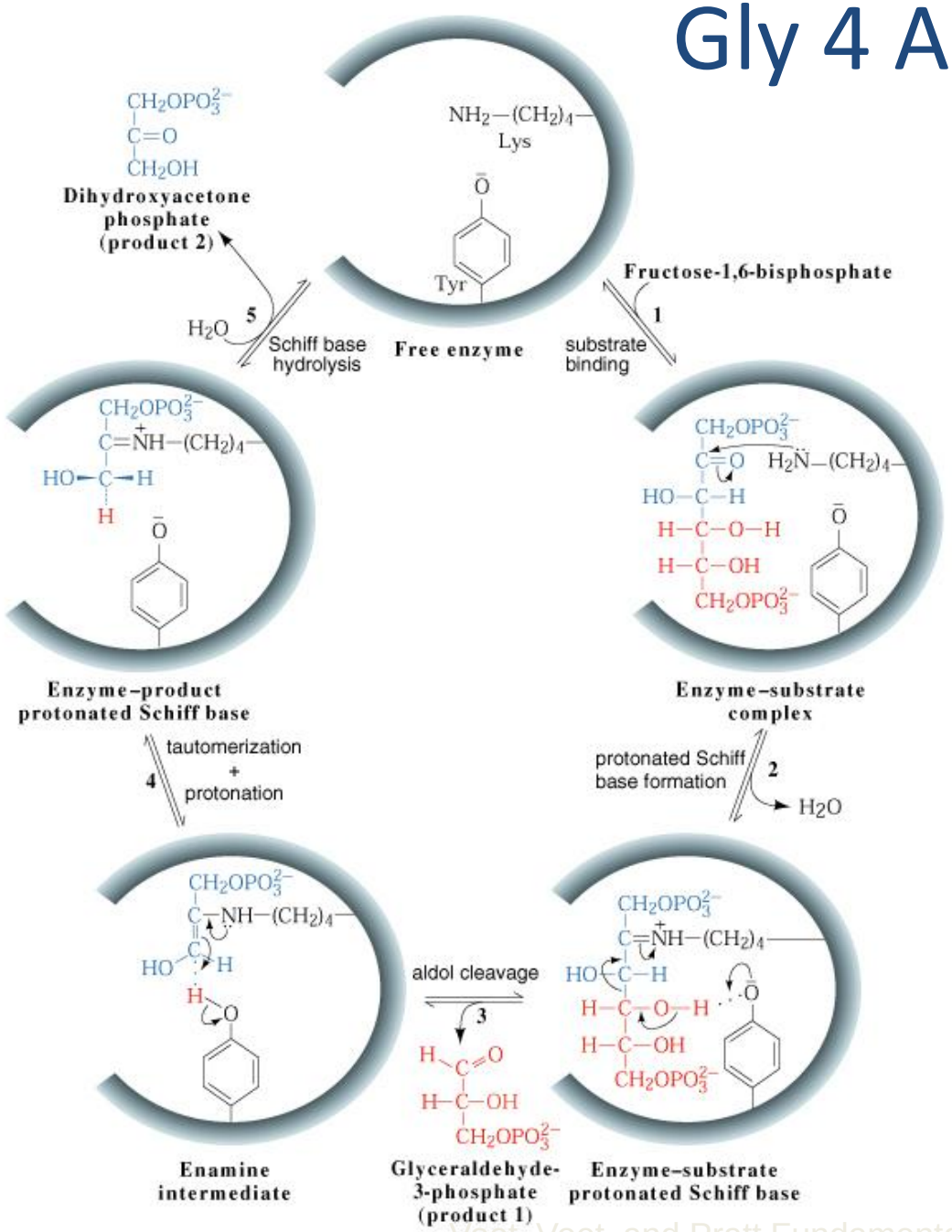
Gly 4 Aldolase



$\Delta G^{o'}$ = +23.8 kJ/mole

The backward reaction (condensation) is spontaneous.

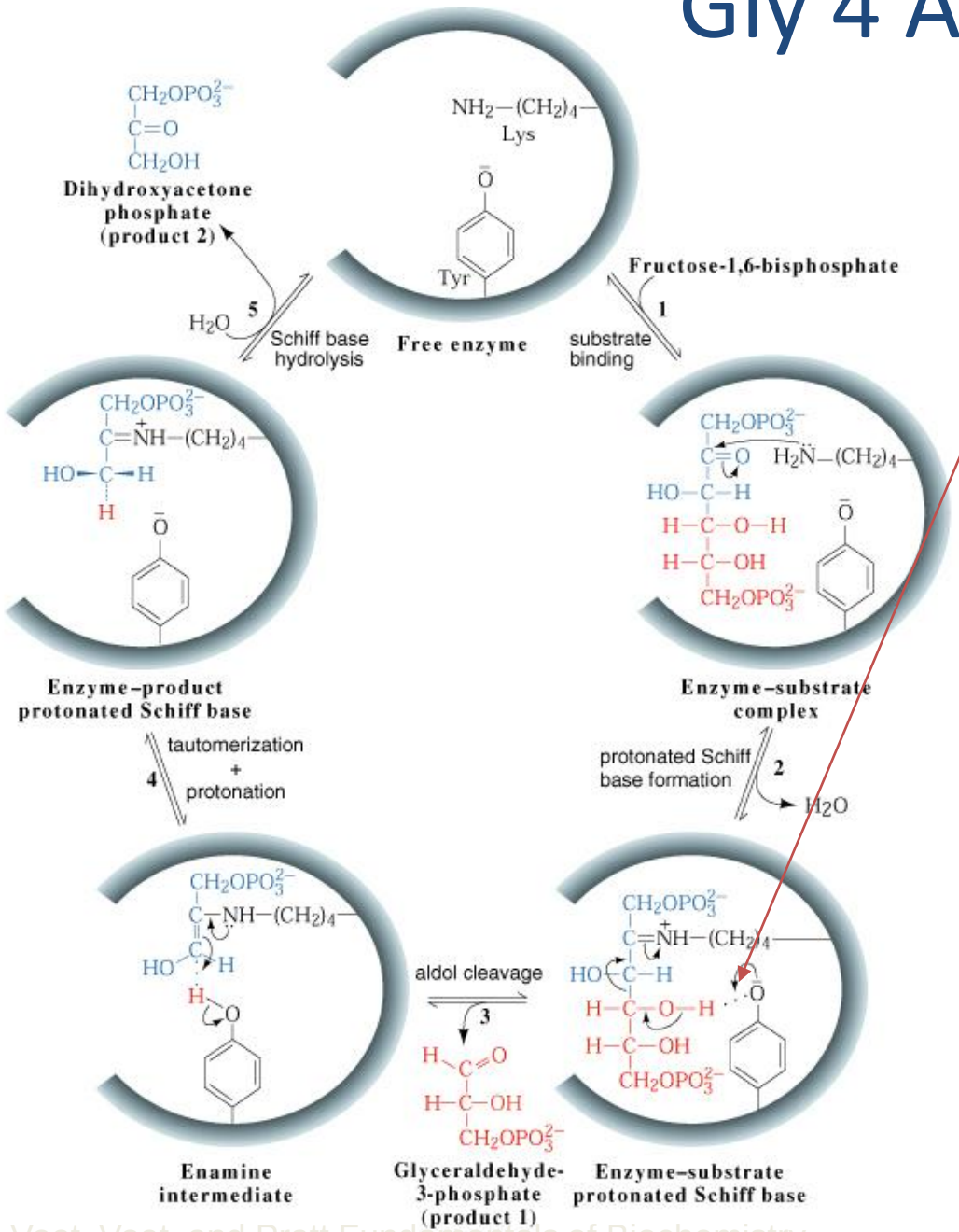
Gly 4 Aldolase Mechanism



Important Themes in Gly 4

1. Schiff Base Linkage:
   - Active site Lysine
   - Covalently binds the substrate through a Schiff base linkage.
   - activates substrate for bond cleavage by acting as an electron sink for negative charge that would otherwise build up on C3

Gly 4 Aldolase Mechanism



2. Base/Acid Chemistry

- tyrosinate at active site initiates C-C bond cleavage by removing a proton from alcohol on C4
- Aldehyde forms on C4, breaking C3-C4 bond
- P_1-GAP, leaves
- tyrosine can donate proton back to C3
- Hydrolysis of bound enamine generated DHAP-P_2. (O^{18} water, incorporated into DHAP)

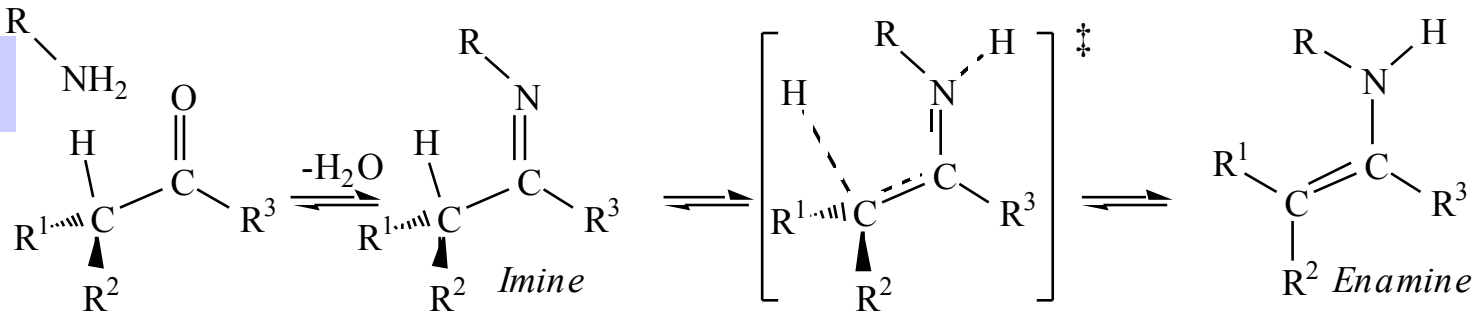
Gly 4: Aldols, and Enols and Enamines

Oh, My!

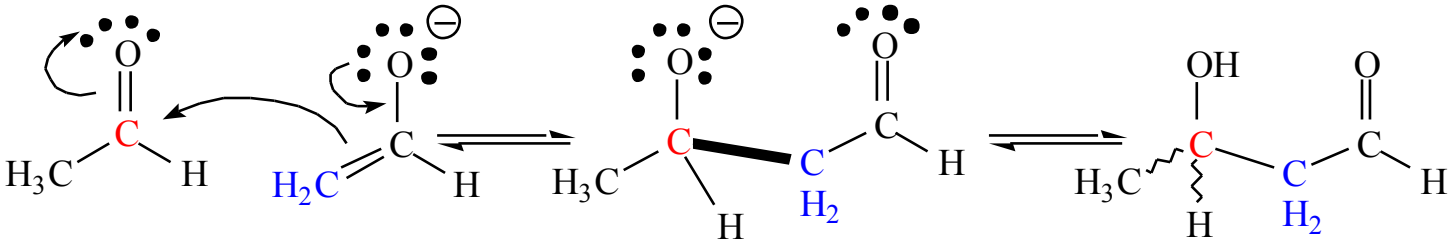
- Enamine formation

SHIFF BASE FORMATION

Lysine



- General Aldol Condensation (Reverse Reaction)



C4 C3

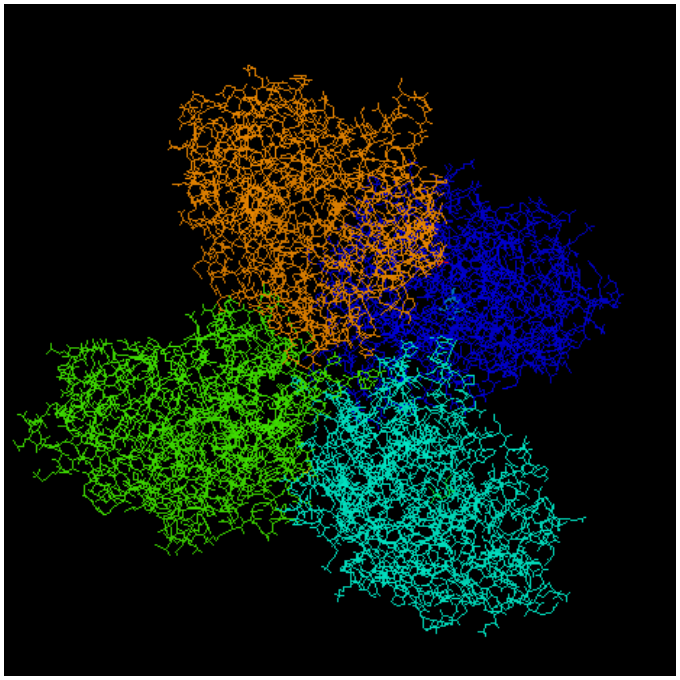
P1 P2

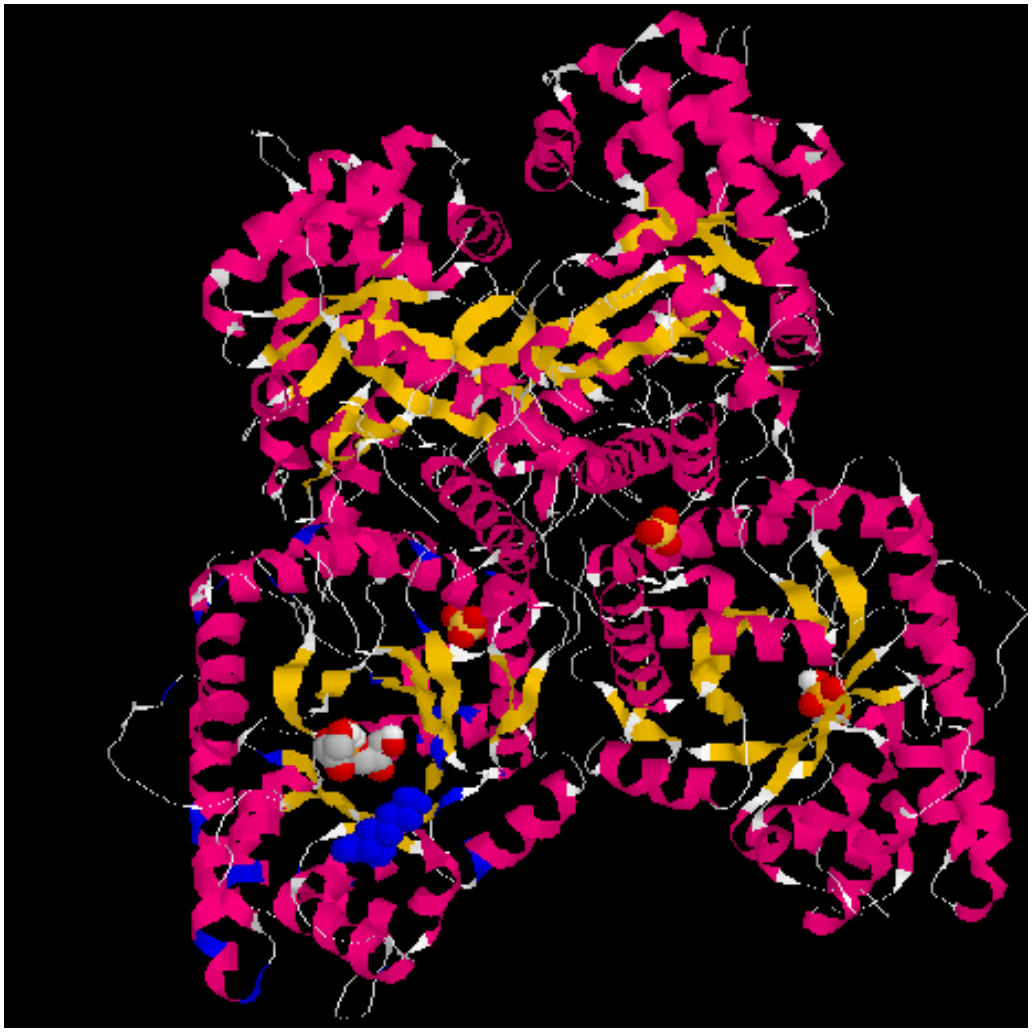
Gly 4 Aldolase Substrate binding

Muscle cells	**K_m**	**S**	**K_m/S**	
[FBP]	100 uM	32 uM	3.1	cleavage
reverse reaction (C-C bond formation)				
[G3P]	1000 uM	3 uM	333	condensation
[DHAP]	2000 uM	50 uM	40	

* FBP binds best, Km/S shows about 1/2 V_{max}
* DHAP, G3P 10X weaker binding, K_m/S shows not at all maximized for this direction.
* Muscle cell has very little of G3P, this pulls the reaction forward.

Gly 4 Aldolase 1ADO





Title Fructose 1,6-Bisphosphate Aldolase From Rabbit Muscle
Classification Lyase Compound
Mol_Id: 1; Molecule: Aldolase;
Chain: A, B, C, D; Ec: 4.1.2.13;
Biological_Unit: Tetramer

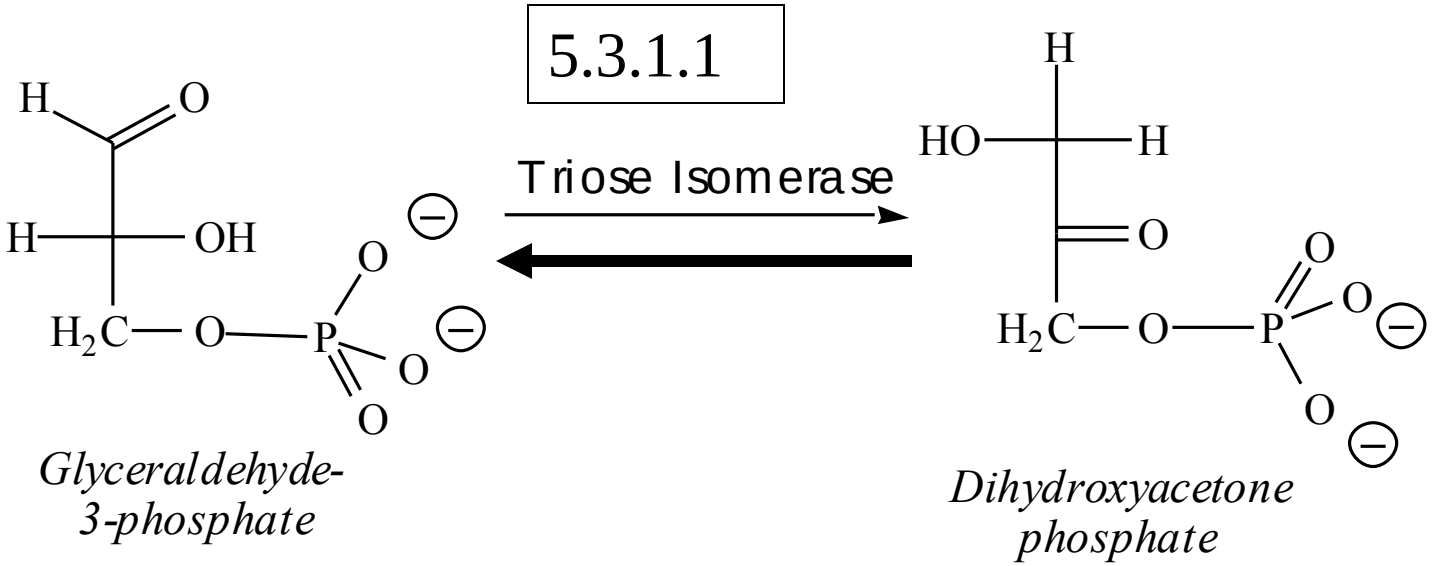
Exp. Method X-ray Diffraction

Active site with Lys41 shown in blue, substrate analogue spacefilled and cpk colored

Gly 4 Aldolase 1ADO

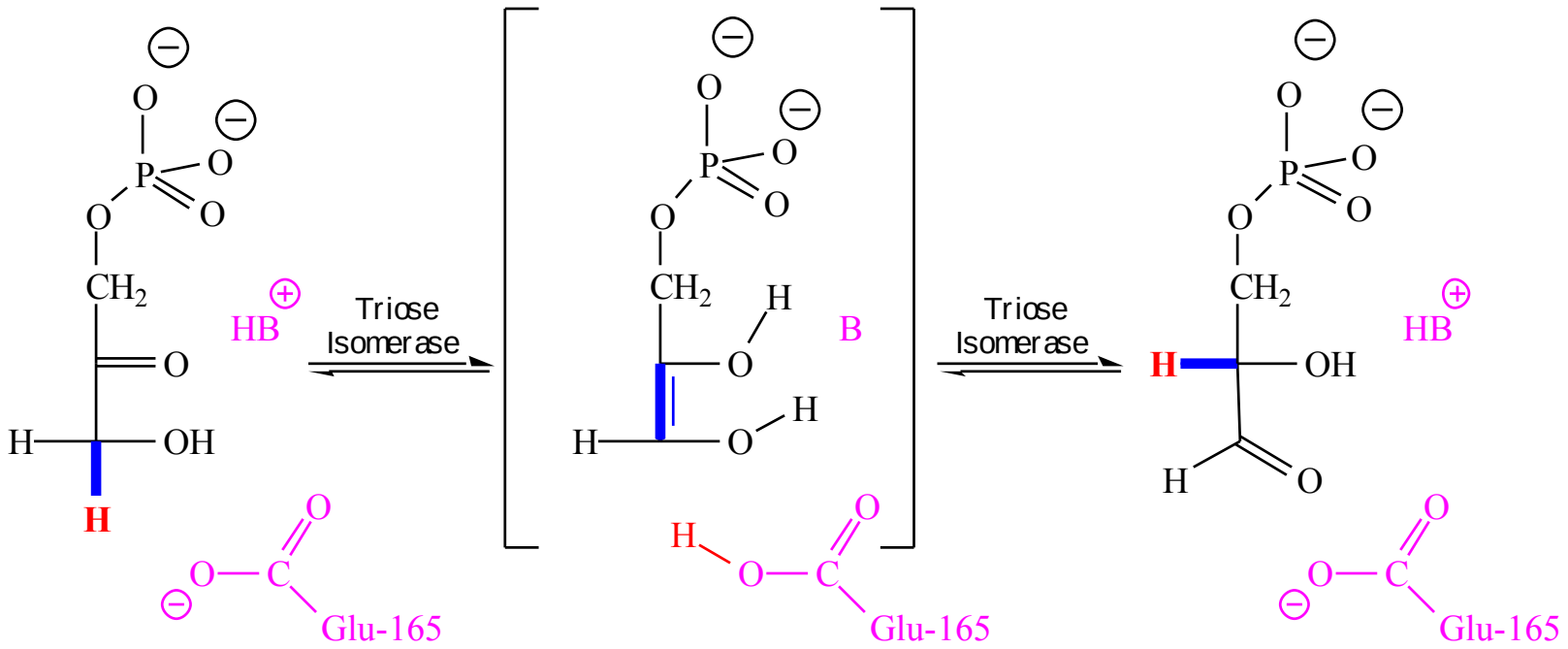
Active site Tyr 301 shown spacefilled in green

Gly 5 Triose Phosphate Isomerase TIM



$\Delta G^{o'}$ ketone to aldehyde = +7.5 kJ/mole prod/react =1/20

Gly 5 TIM Mechanism



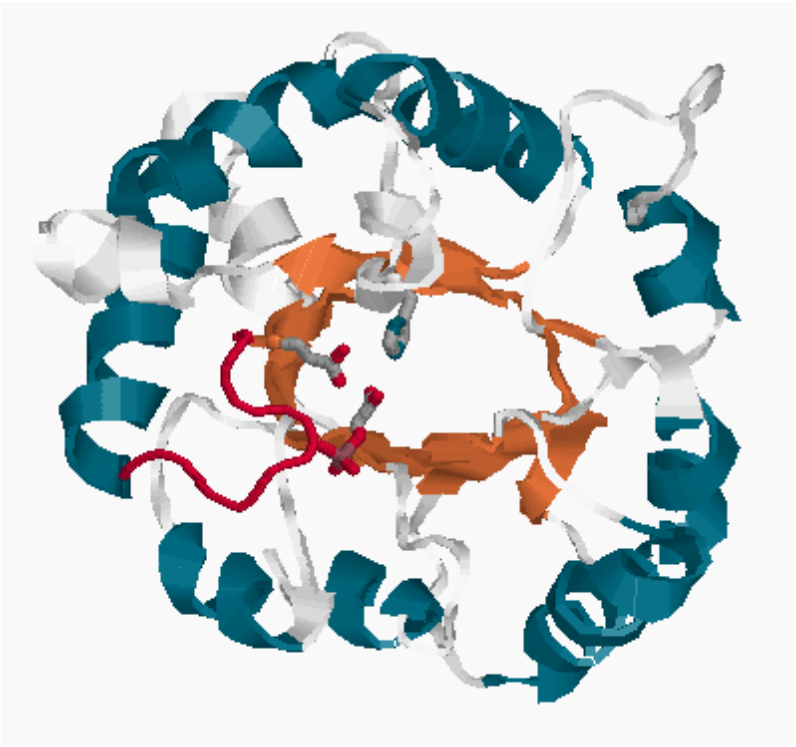
- Enol formation requires both protonation and deprotonation
- Two groups involved in proton shuttles: Glu-165 and His-95.

Gly 5 TIM Kinetics for DHAP

- k_{cat} = 2.0 x 10^5 s^{-1} (ΔG^{tt} = 10.4 kcal/mole)
  - modest catalysis
- K_m = 8.7 x 10^{-4} M
  - enzyme binds just well enough (0.87 mM)
- k_{cat}/Km = 2.4 x 10^8 $M^{-1}s^{-1}$
  - virtually the highest possible for bimolecular process of E + DHAP = E + GAP, ***TIM is the perfect enzyme***
- Close to saturation?
  - [S] = [DHAP] = 50 uM
  - Km = 870 uM
  - Km/S = 17/1 enzyme not working at V_{max}, can go faster if necessary

Gly 5 TIM structure

- Enzyme Motion
  - When the enzyme binds its substrate the loop closes over the active site, shielding the substrate from water. The loop appears to close as a rigid lid, stabilized by internal hydrogen bonds. Its motion involves movements of alpha carbons of up to 8 A. The closure involves the filling of a cavity near the base of the helix to which the loop is connected and the formation of new hydrogen bonds and contacts.
  - Structures 2YPI , 3TIM , 6TIM



Gly 5 TIM Structure 2YPI

Crystallographic Analysis Of The Complex Between Triosephosphate Isomerase and 2-Phosphoglycolate At 2.5 Å

Classification
Isomerase (Intramolecular Oxidoreductse)
Compound Triose Phosphate Isomerase (TIM) (E.C. 5.3.1.1) Complex With 2-Phosphoglycolic Acid
Exp. Method X-ray Diffraction

Gly 5 TIM Structure 2YPI

Glu 165 that acts as a base to abstract a proton is shown in blue at right

His95 that participates in acid/base mechanism shown in yellow at left.

Phase II

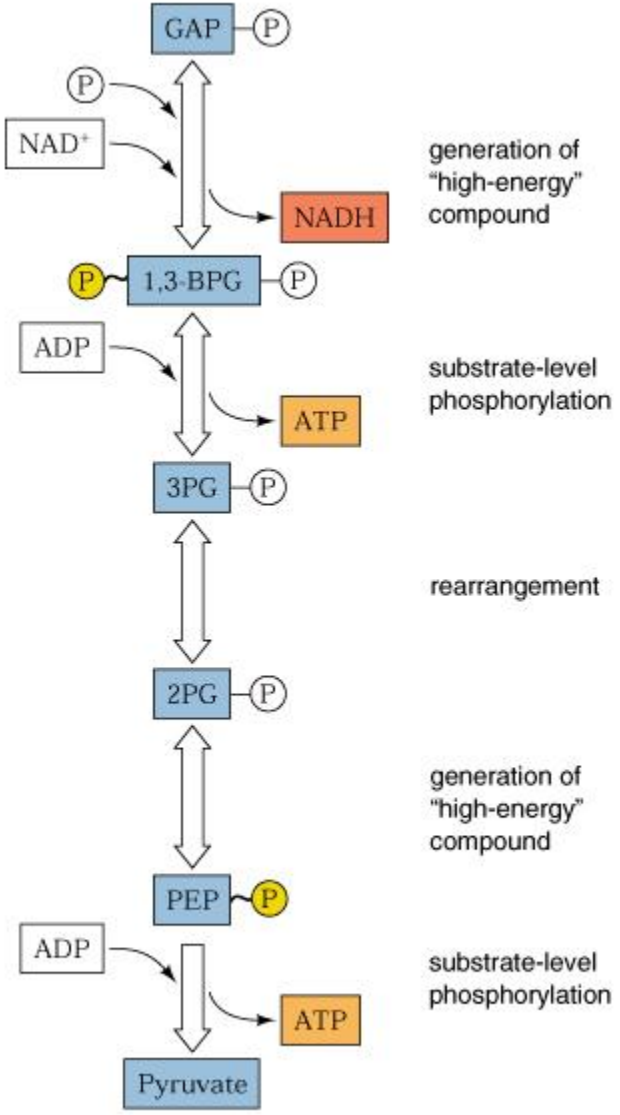
$2GAP + 2NAD^{+} + 4\ ADP + 4\ HPO_4^{-2}$

$\dashrightarrow 2\ pyruvate + 2\ NADH + 4\ ATP + 6\ H_2O$

Cashing In, here we see a production of 2 NADH and when we subtract the 2 ATP invested from 4 ATP above, *net* 2 ATP

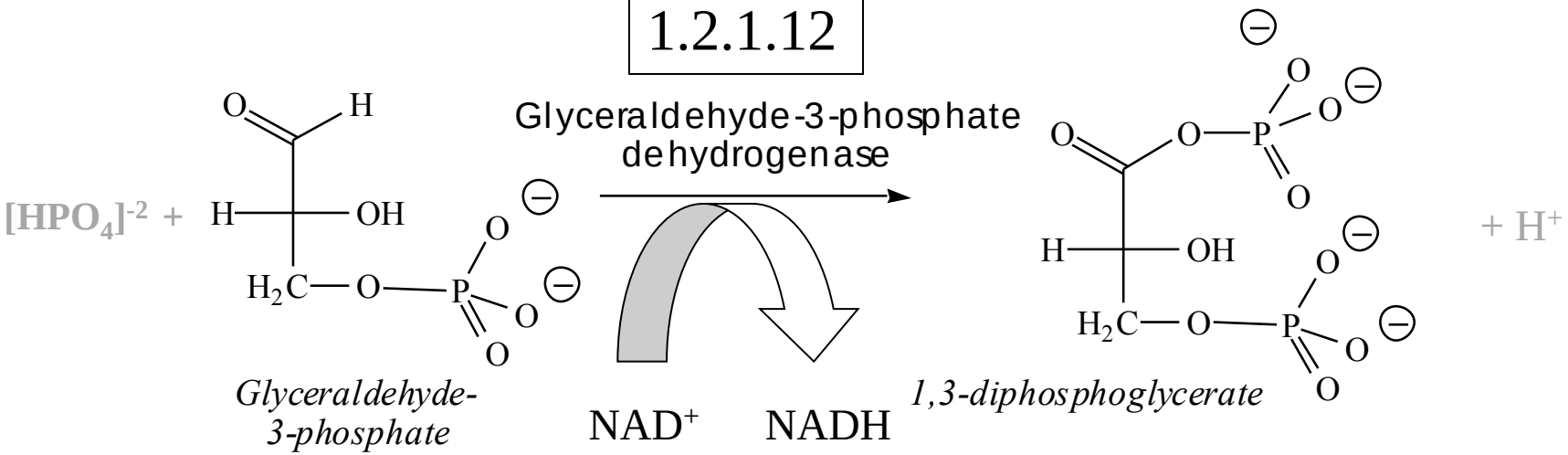
Note that reaction above isn't charge or mass balanced, and protons aren't accounted for directly, some reactions will generate.

6 waters produced, 2 come from substrate in gly 9 and 4 come from gly 7 and gly 10 ($ADP^{-4} + HPO_4^{-2} = ATP^{-6} + H_2O + H^{+}$)

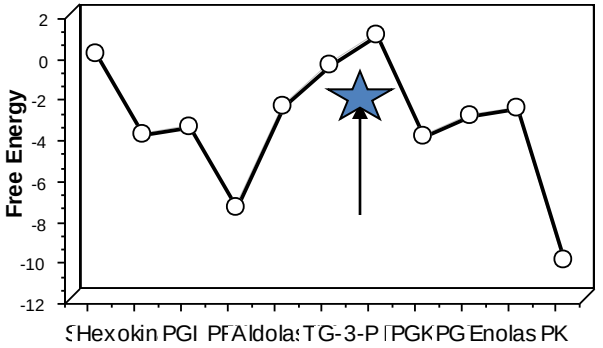


Gly 6: Glyceraldehyde-3-Phosphate Dehydrogenase:

1.2.1.12



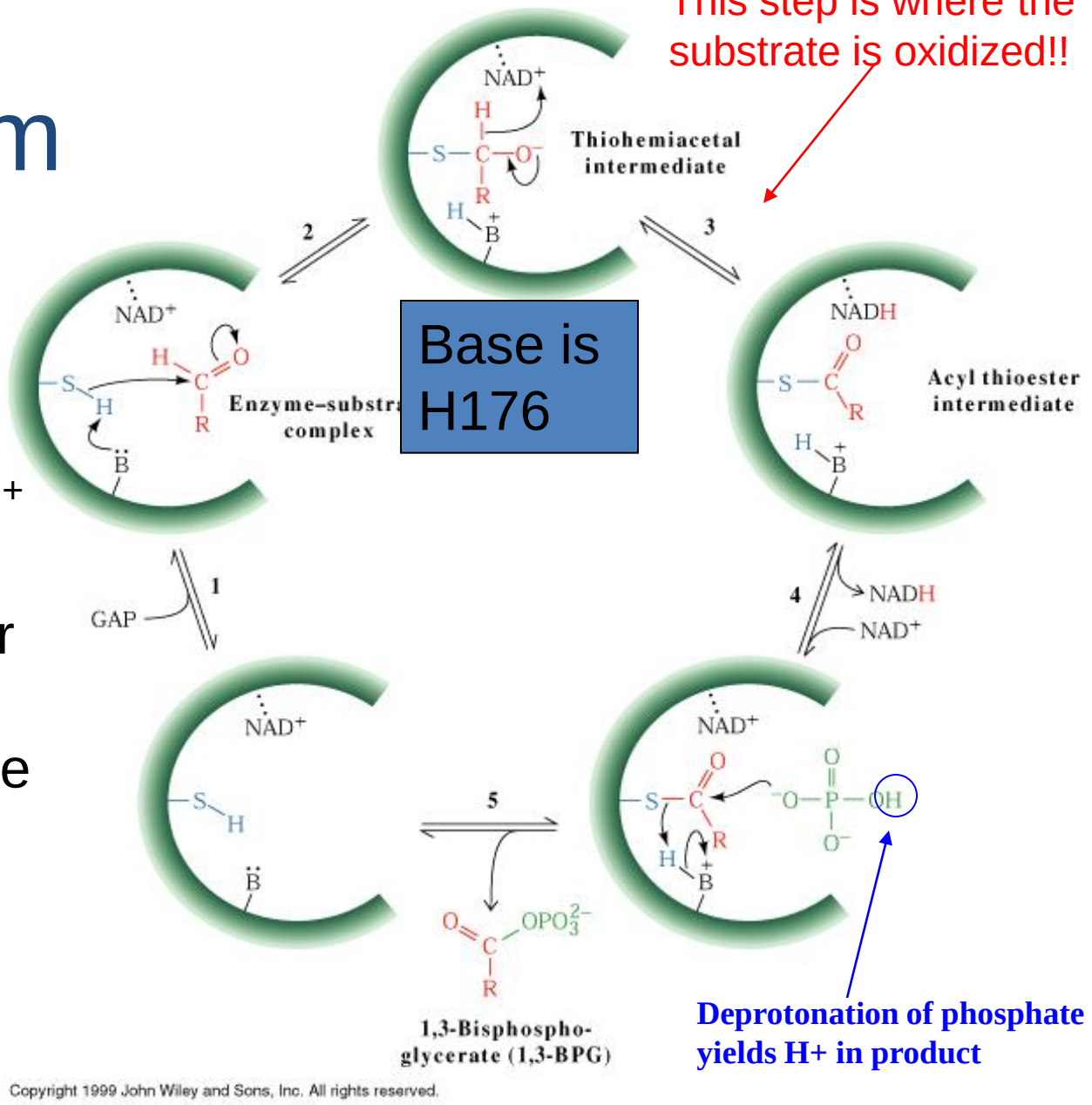
- Note: overall oxidation
- Exergonic oxidation of aldehyde drives reaction
- Cofactor: NAD^+



$\Delta G^{o'}$ = +6.3 kJ/mole

Gly 6: Mechanism

*C149 attacks GAP, H176 grabs proton
*thiohemiacetal intermediate
* hydride shift to NAD^+ GAP oxidized
* second acylthioester intermediate
* NADH release is rate limiting
*second NAD+ binds
* P_i is 2nd substrate
*1,3 BPG released



Gly 6: Saturation? Km and [S]

GAP + NAD^+ + P_i = 1,3 DPG + NADH

	Km	[S]	Km/[S]	
GAP	70 uM	3 uM	23	less than ½ V_{max}
NAD+	46 uM	600 uM	0.08	sat'd w NAD+
Pi	high	2,000 uM	>10	weak binding

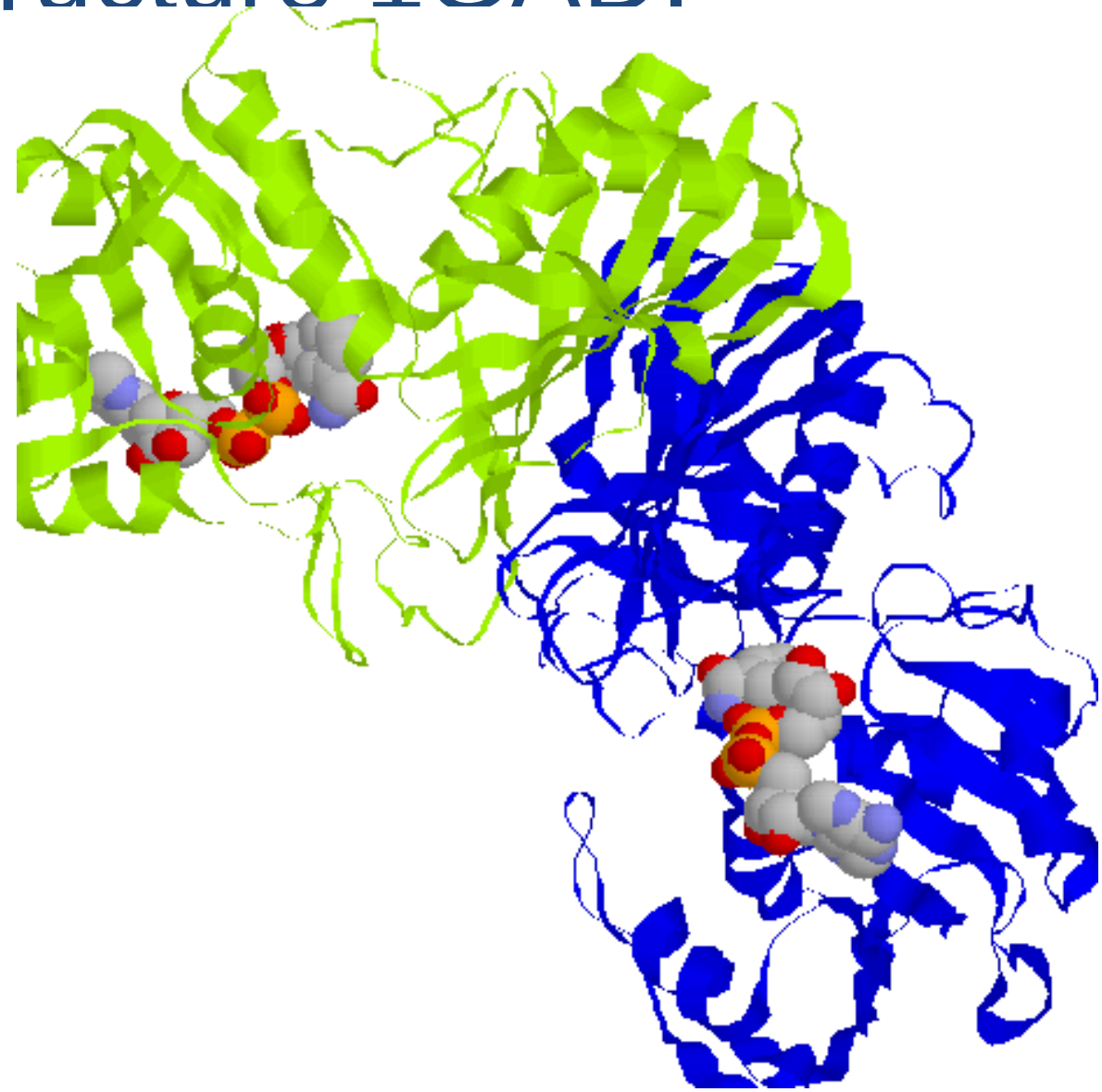
- Km/S<< 1 predicts that gly6 is saturated w NAD+ and not sensitive to changes in it, or controlled by its concentrations
- GAP and Pi both have Km/S >1, both are >10 for very different reasons. GAP has good binding but a low concentration of substrate. Pi has horrible binding, but a high concentration of substrate. Since the binding won't change, **we say that reaction is controlled by GAP** because small changes in its concentration will dramatically alter the rate.

Gly 6:Structure 1GAD.

* Dimer structure from *e.coli*

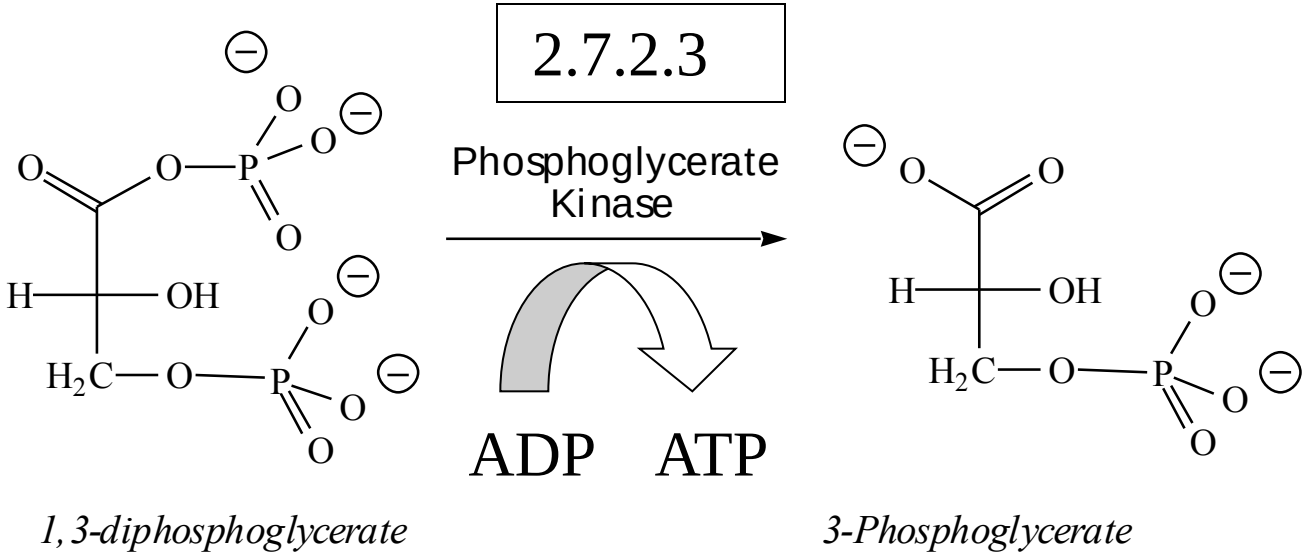
* NAD^+ binding in Rossman fold

* Pos. Cooperativity Hill 2.3



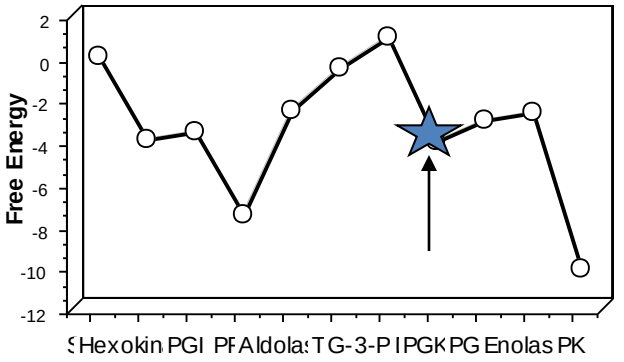
Gly 7: Phosphoglycerate Kinase

First ATP Generation



- Very exergonic , ATP generated
- Only monomeric enzyme on pathway
- Closely associated with Gly6

$\Delta G^{o'}$ = -18.5 kJ/mole

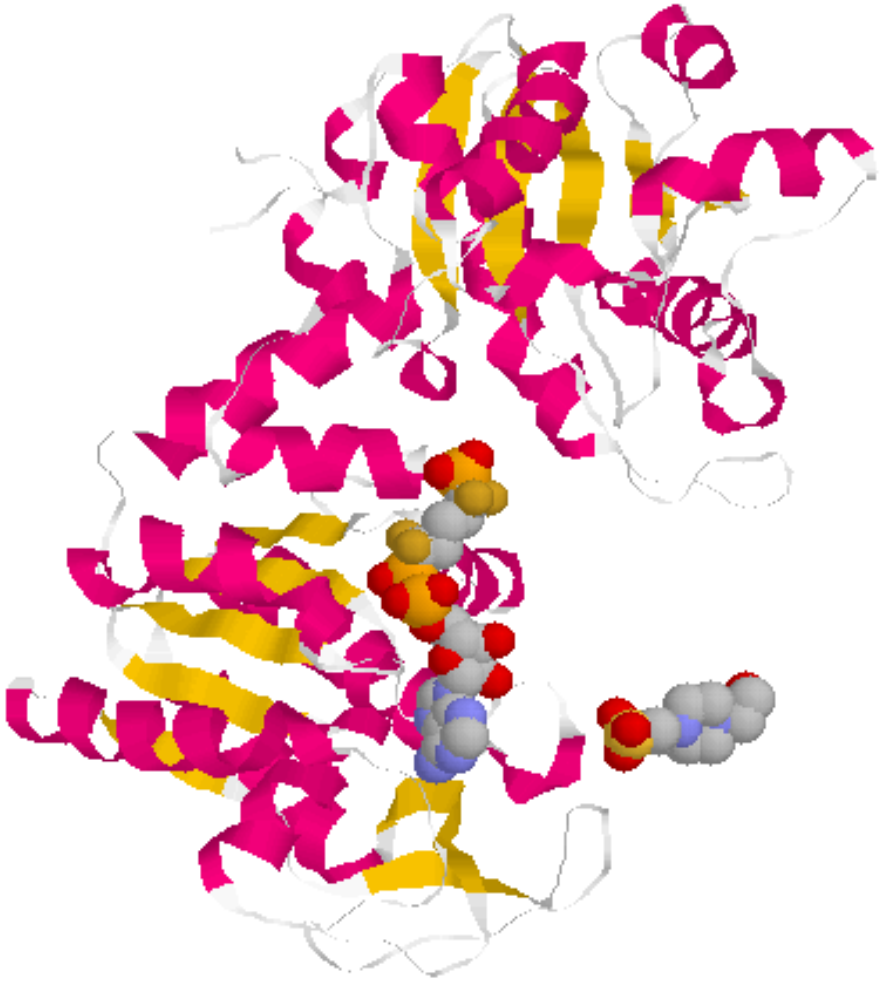


Gly 7: Structure 16PK from *Trypanosoma brucei*

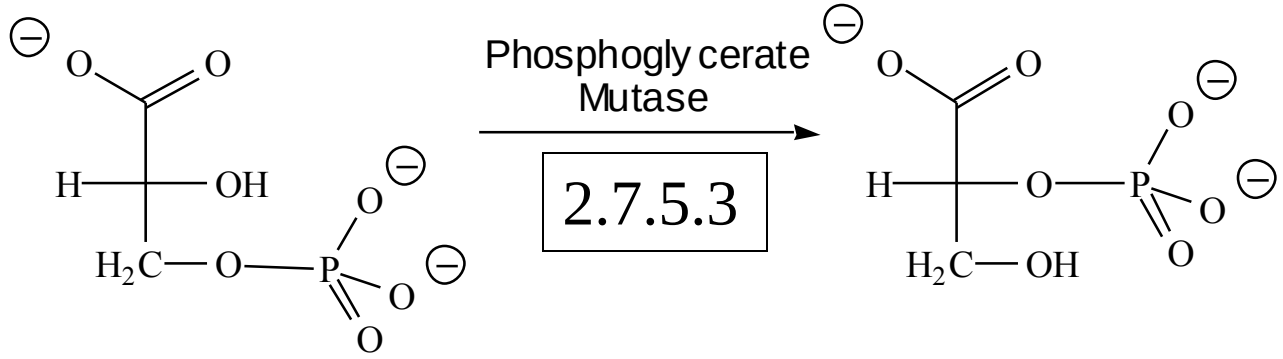
* ADP binding site looks like NADH binding site, here crystallized with NADH analogue

* ADP binds tightly, more like cofactor, Km/S = 0.05

* 1,3 DPG Km/S = 9

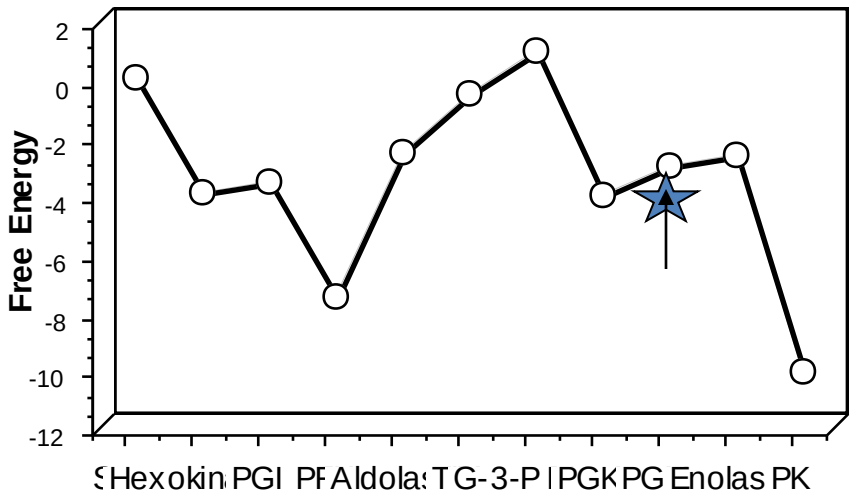


Gly 8: Phosphoglycerate Mutase



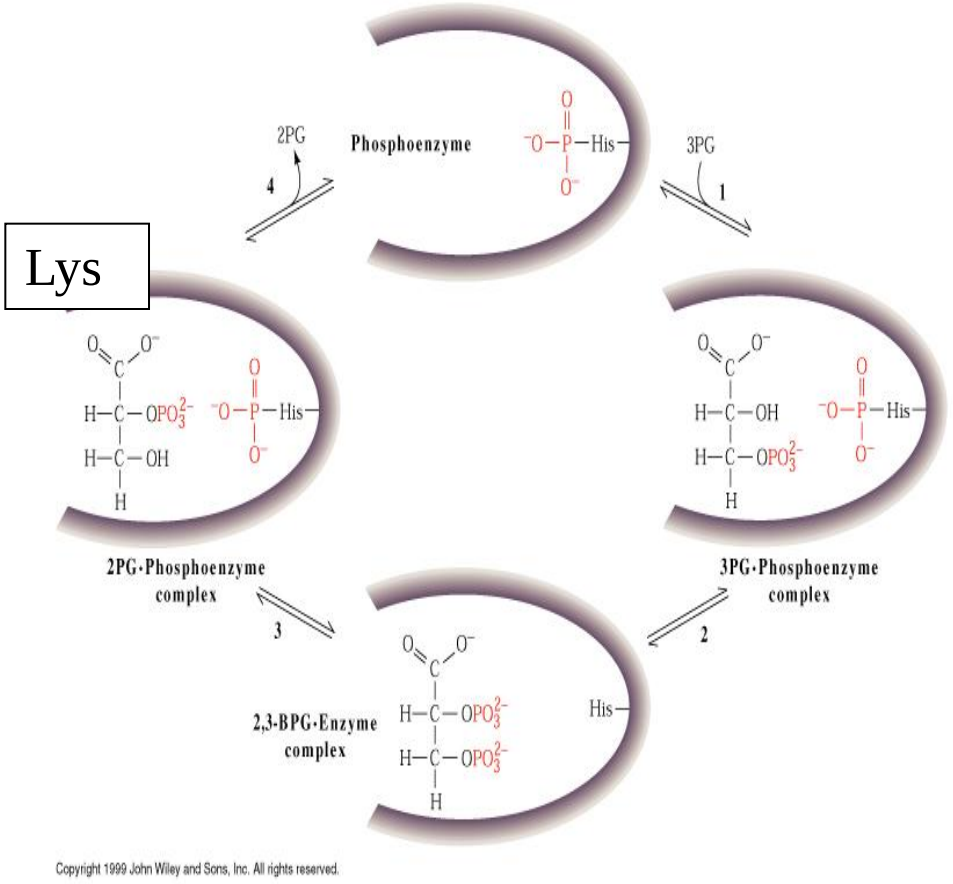
3-Phosphoglycerate *2-Phosphoglycerate*

$\Delta G^{o'}$ = +4.4 kJ/mole



Gly 8: PGM Mechanism

*Base (Lys?) interacts with carboxylic acid of 3PG for binding
* Active enzyme phosphor-ylated by 2,3-DPG
*His His duo at active site
*Km varies from 240 uM in brain to 5000 uM in muscle. (nature of base?)
*[S] 3PG 40-60 uM
*Km/S = 6-80, E not sat'd with S, room to speed up.



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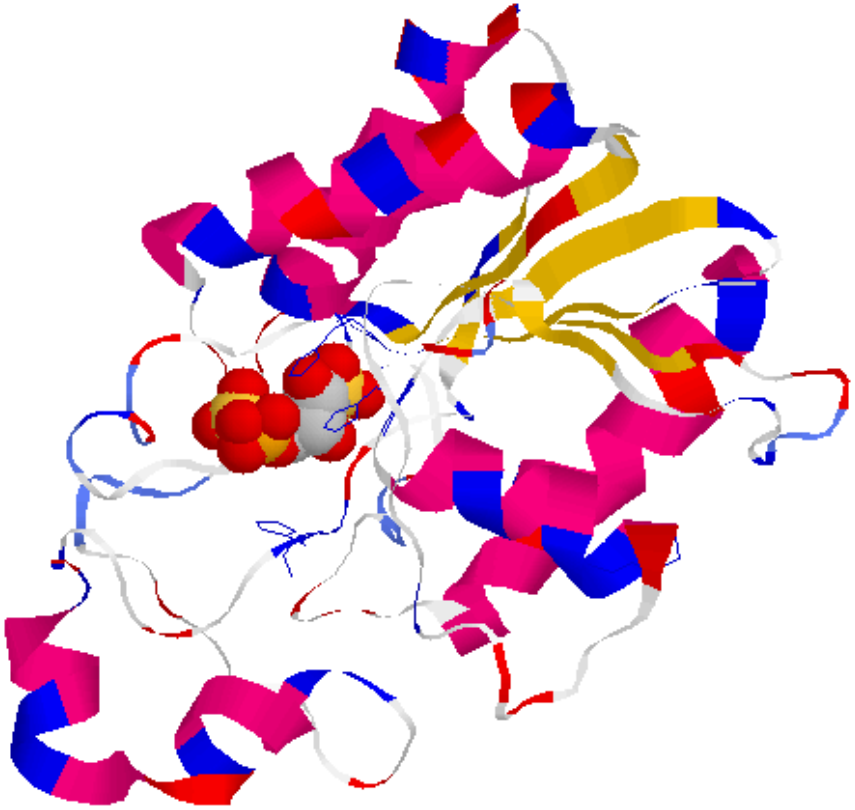
Gly 8: 3PGM structure from *Schizosaccharomyces pombe*



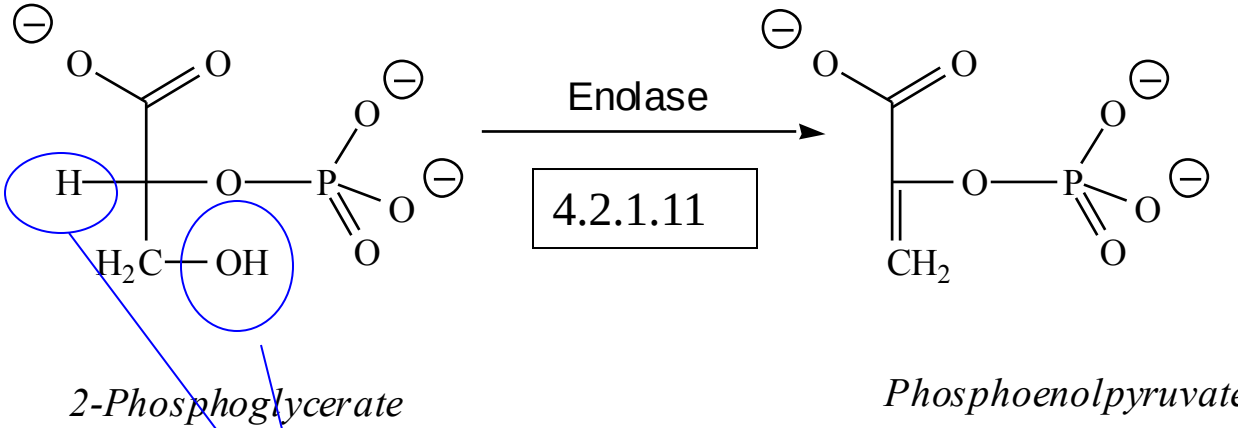
- Gly 8 has a relatively simple structure with a beta twist and helical unit, 3PG binds at interface.
- Two his 4 Å apart shown in blue wireframe
- His adjacent to C2 has Pi, His adjacent to C3 extracts Pi from C3 while OH on C2 extracts Pi from Pi-His

Gly 9: Enolase

Second "High-Energy" Intermediate Formation

Is there next oxidation or reduction of substrate in Gly 9?

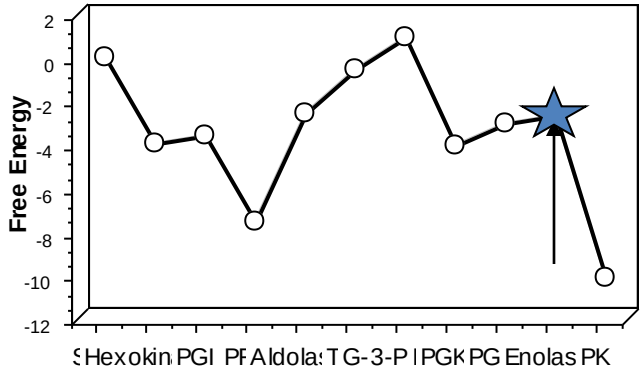
Answer No, because C2 becomes oxidized (0 to +1) while C3 becomes reduced (-1 to –2) so no net electron flow.



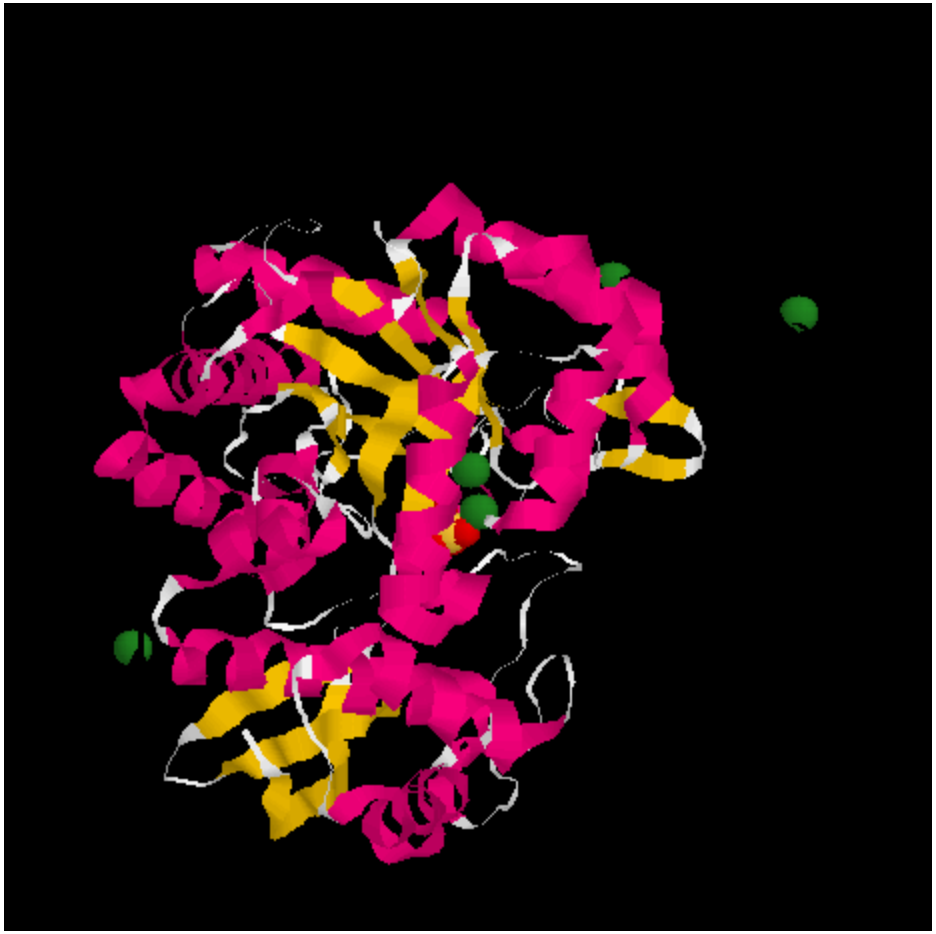
Dehydration to mobilize phosphate accompanied by shift from sp^3 to sp^2 geometry on 2PG

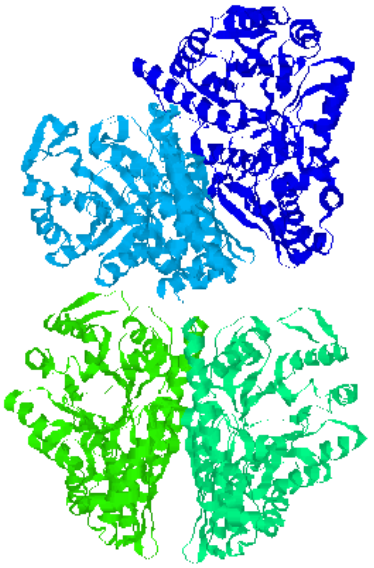
Km 50 uM, S 5 uM, Km/S = 10

$\Delta G^{o'}$ = +7.5 kJ/mole



Gly 9: 1E9I Structure from *E. coli*





* exists as tetramer, positive cooperativity

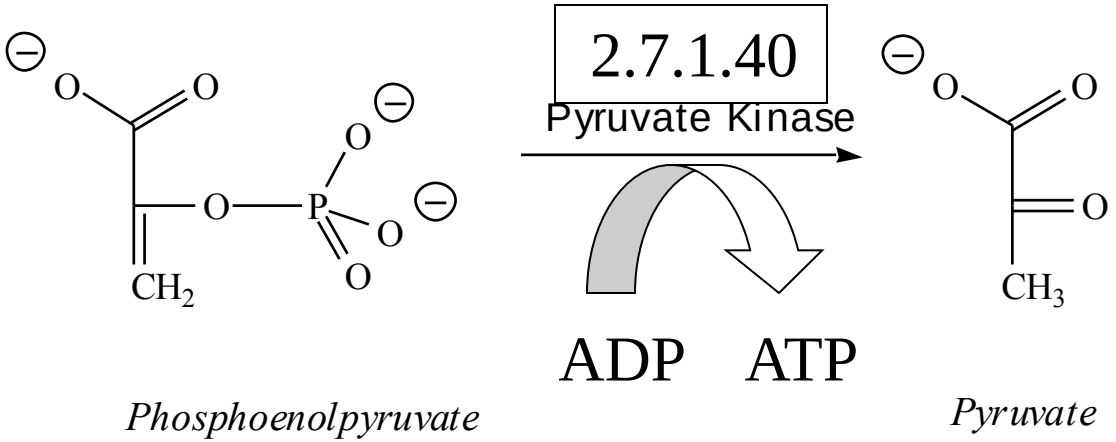
* Mg(II) must bind first, image shows 2 Mg(II) and Pi shown bound at active site.

Gly 10: Pyruvate Kinase

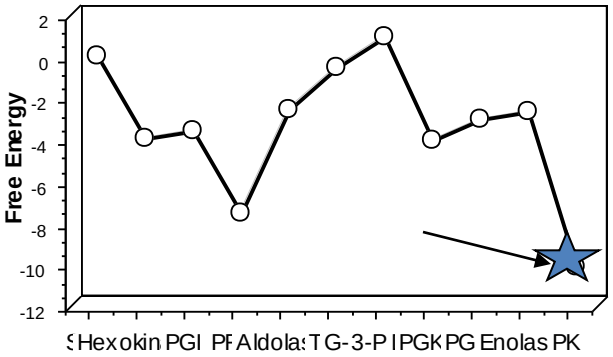
Second ATP Generation

Is there next oxidation or reduction of substrate in Gly 10?

Answer No, because C2 becomes further oxidized (+1 to +2) while C3 becomes further reduced (–2 to -3) so no net electron flow.

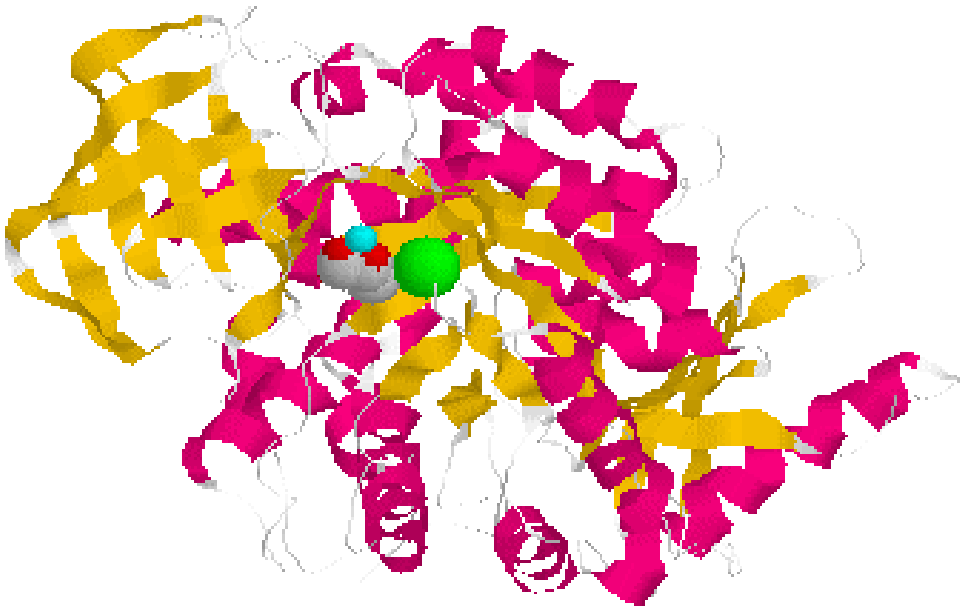


Transfer of P to ADP, is slow, conversion from enol to ketone is rapid



$\Delta G^{\circ\prime} = -31.4$ kJ/mol

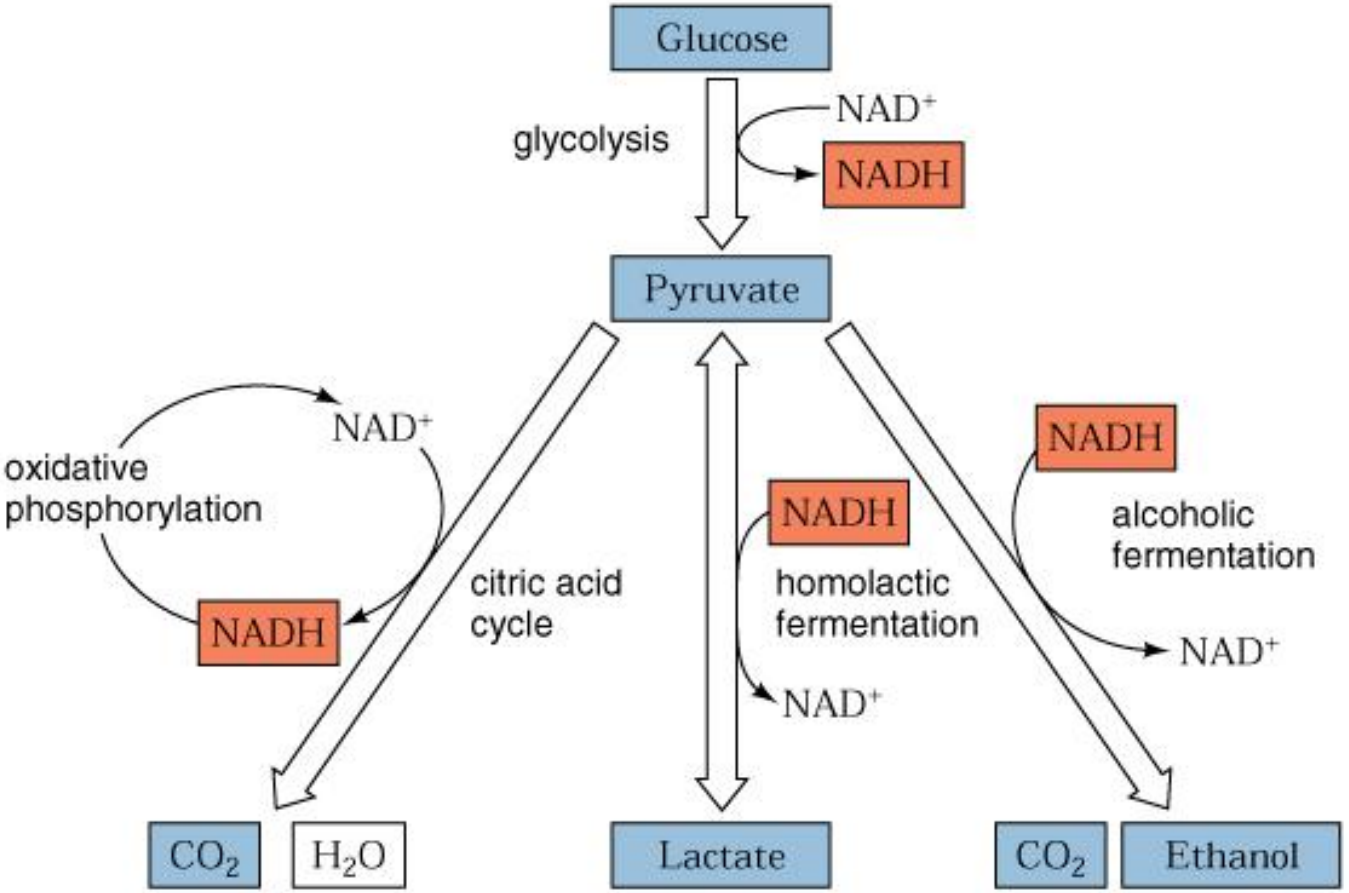
Gly 10 1PKN from rabbit muscle



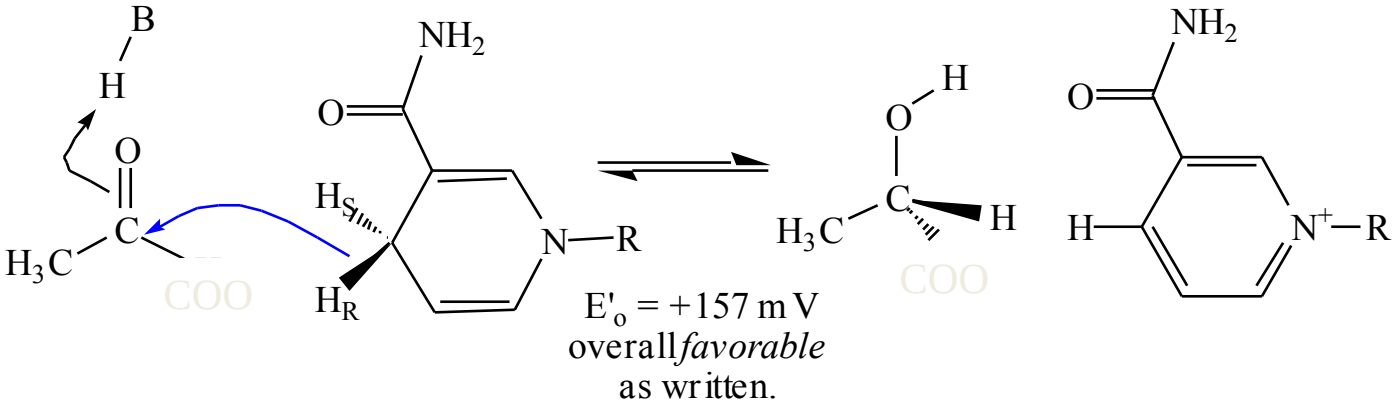
PDB File

- Monomer rich in beta barrels with 3 domains, has both a K^{+} and a Mg^{+2} at active site.
- Exists as tetramer with positive coop 2.8 (only monomer shown here)
- Allosteric effectors
  - FBP (+)
  - ATP (-)
- R and T states
- isozymes L and M

Fates of Glucose: Fermentation



LDH Mechanism: NADH redox



•redox potential of NADH varies in different enzymes:

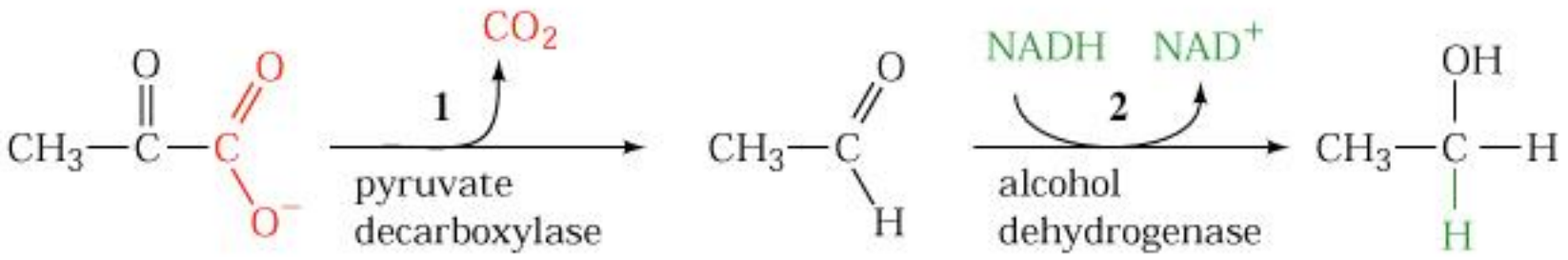
• transfer of the proH_R or proH_s hydride to substrate depends on enzyme class

• binding site selects conformation of the nicotinamide ring and only one stereoselected H is transferred (for reduction) or added (for oxidation).

•His 195 donates a proton to ketone, accepts a proton from alcohol

•Both His 195 and Arg 171 interact electrostatically to orient carboxyllic acid of pyruvate in enzyme active site

Fermentation: Alcohol



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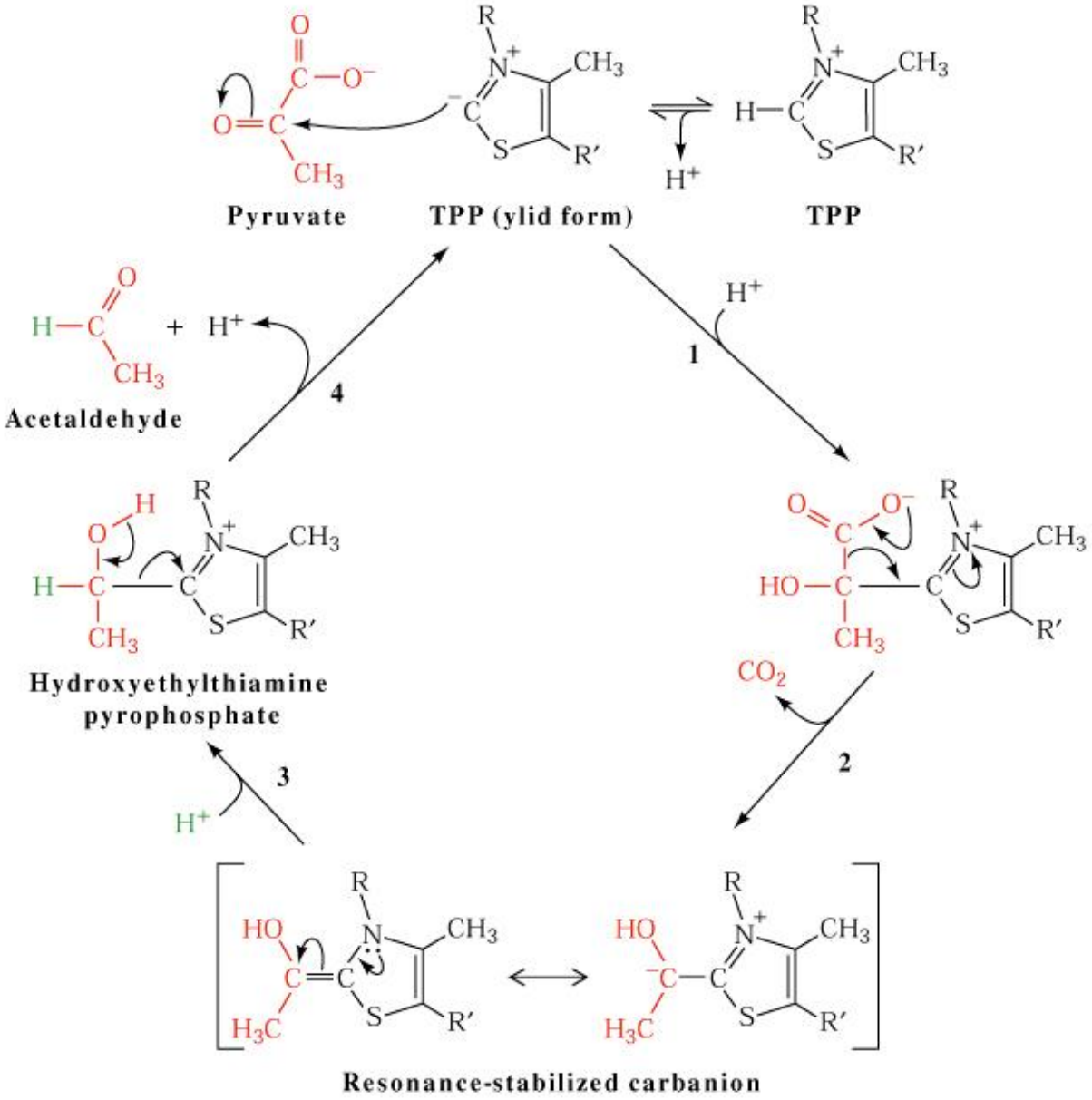
Pyruvate Decarboxylase Mechanism

Thiamine pyrophosphate, coenzyme

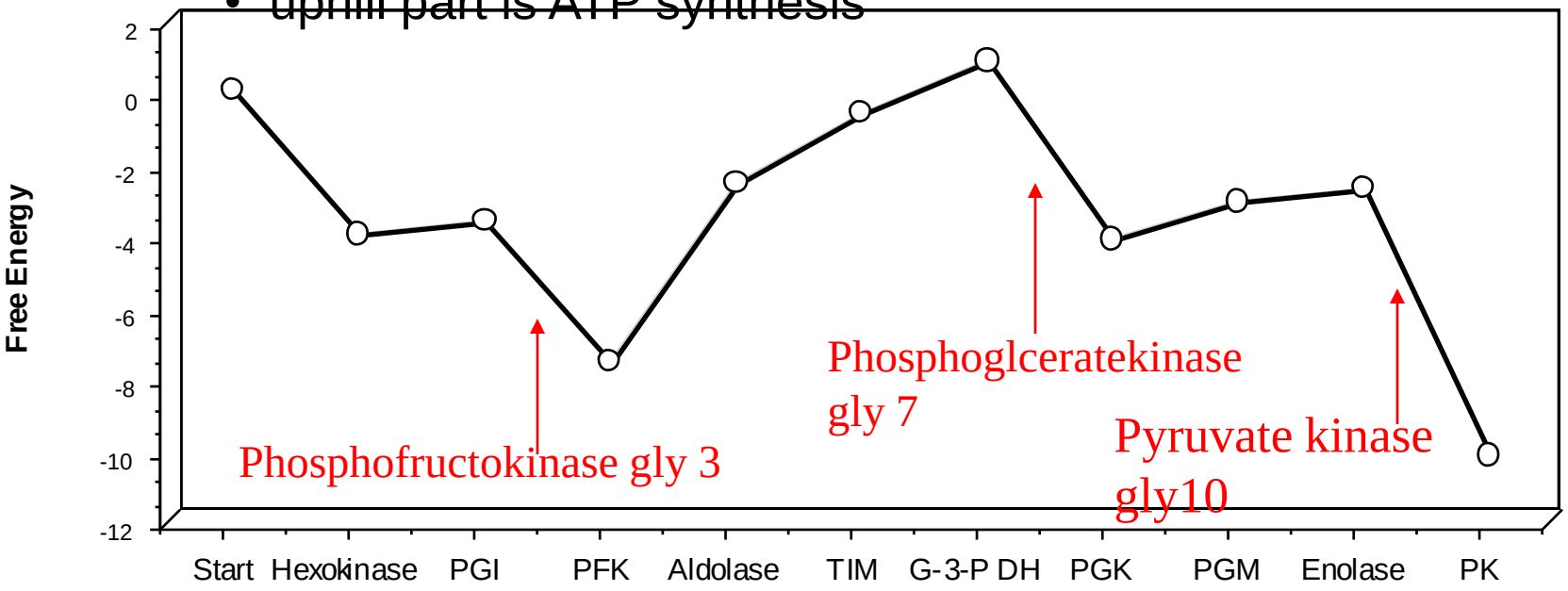


Control of Glycolysis

- Energy Coupling (review phosphoryllation potential slide 5)
  - Gly 3? driven by ATP hydrolysis
    - uphill part is phosphoryllation of sugar
  - Gly 7? driven by BPG hydrolysis
    - uphill part is ATP synthesis
  - Gly 10? driven by PEP hydrolysis
    - uphill part is ATP synthesis

Major control point

Not likely control since it is the last step in glycolysis



Free Energy of Hydrolysis

Table 13-2. **Standard Free Energies of Phosphate Hydrolysis of Some Compounds of Biological Interest**

Compound	$\Delta G^{\circ\prime}$ $(kJ \cdot mol^{-1})$
Phosphoenolpyruvate	−61.9
1,3-Bisphosphoglycerate	−49.4
Acetyl phosphate	−43.1
Phosphocreatine	−43.1
PP_i	−33.5
ATP (→ AMP + PP_i)	−32.2
ATP (→ ADP + P_i)	−30.5
Glucose-1-phosphate	−20.9
Fructose-6-phosphate	−13.8
Glucose-6-phosphate	−13.8
Glycerol-3-phosphate	−9.2

Source: Jencks, W.P., *in* Fasman, G.D. (Ed.), *Handbook of Biochemistry and Molecular Biology* (3rd ed.), Physical and Chemical Data, Vol. I, pp. 296–304, CRC Press (1976).

20 mM in heart

PFK--Committed Step: Allosteric Control

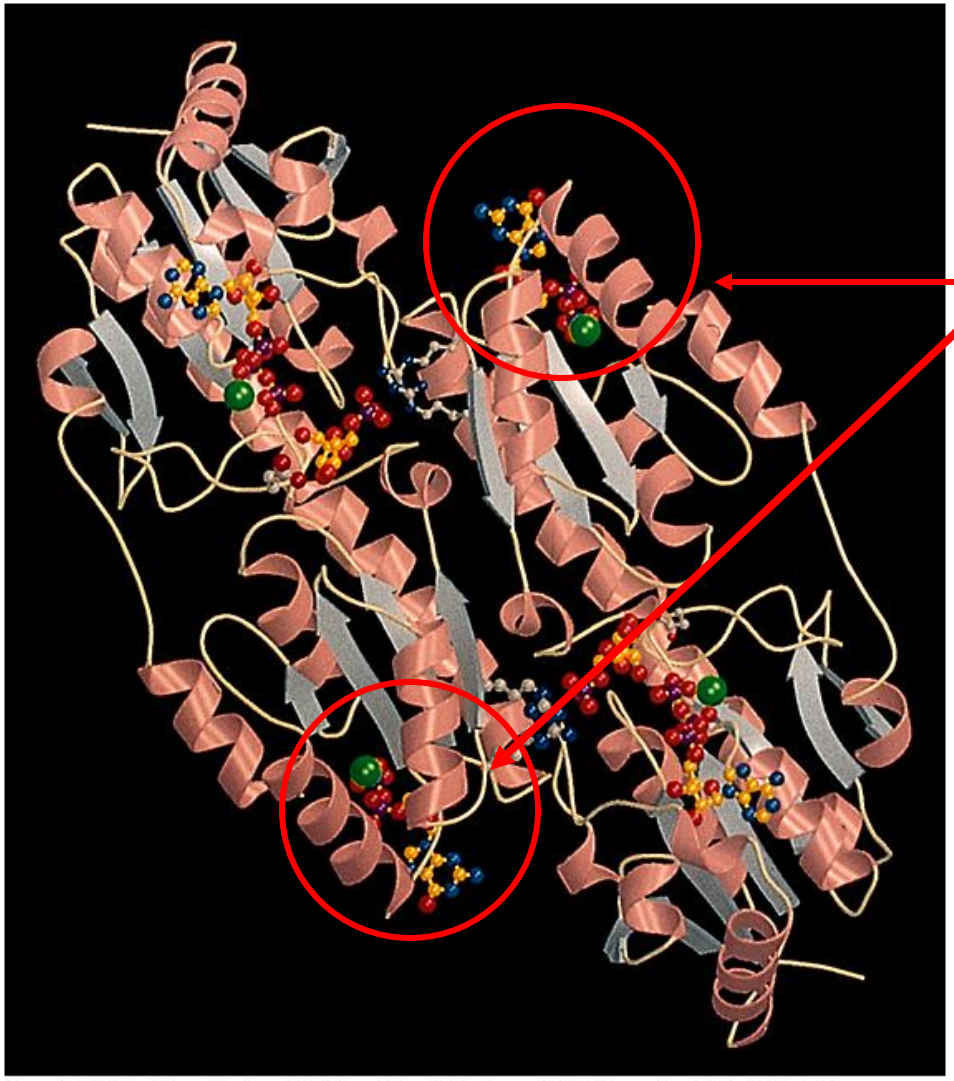


Figure 14-21. The X-ray structure of PFK from *E. coli*. [Courtesy of Philip Evans, Cambridge University.]

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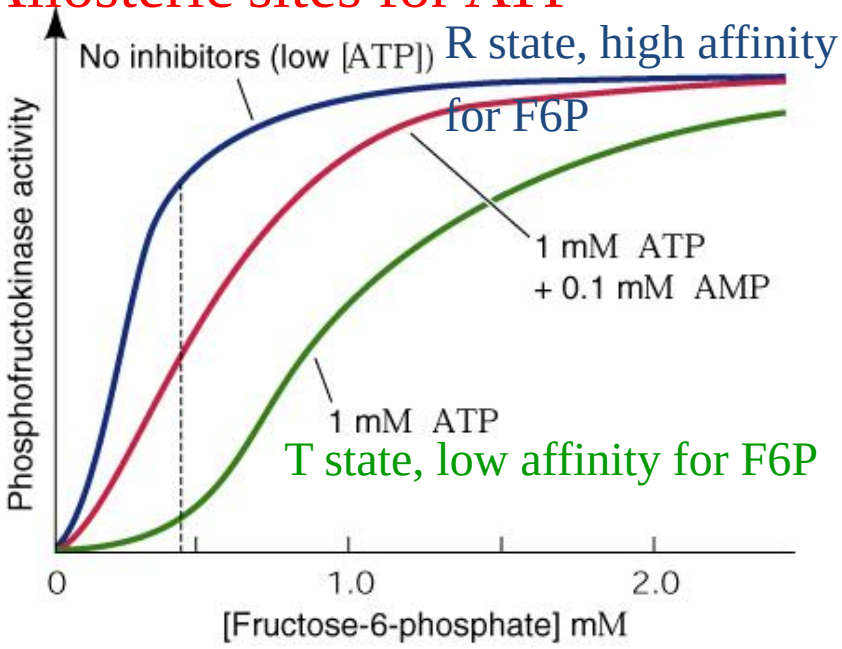
Dimer of PFK shown

Substrate binding sites in center

Allosteric sites for ATP

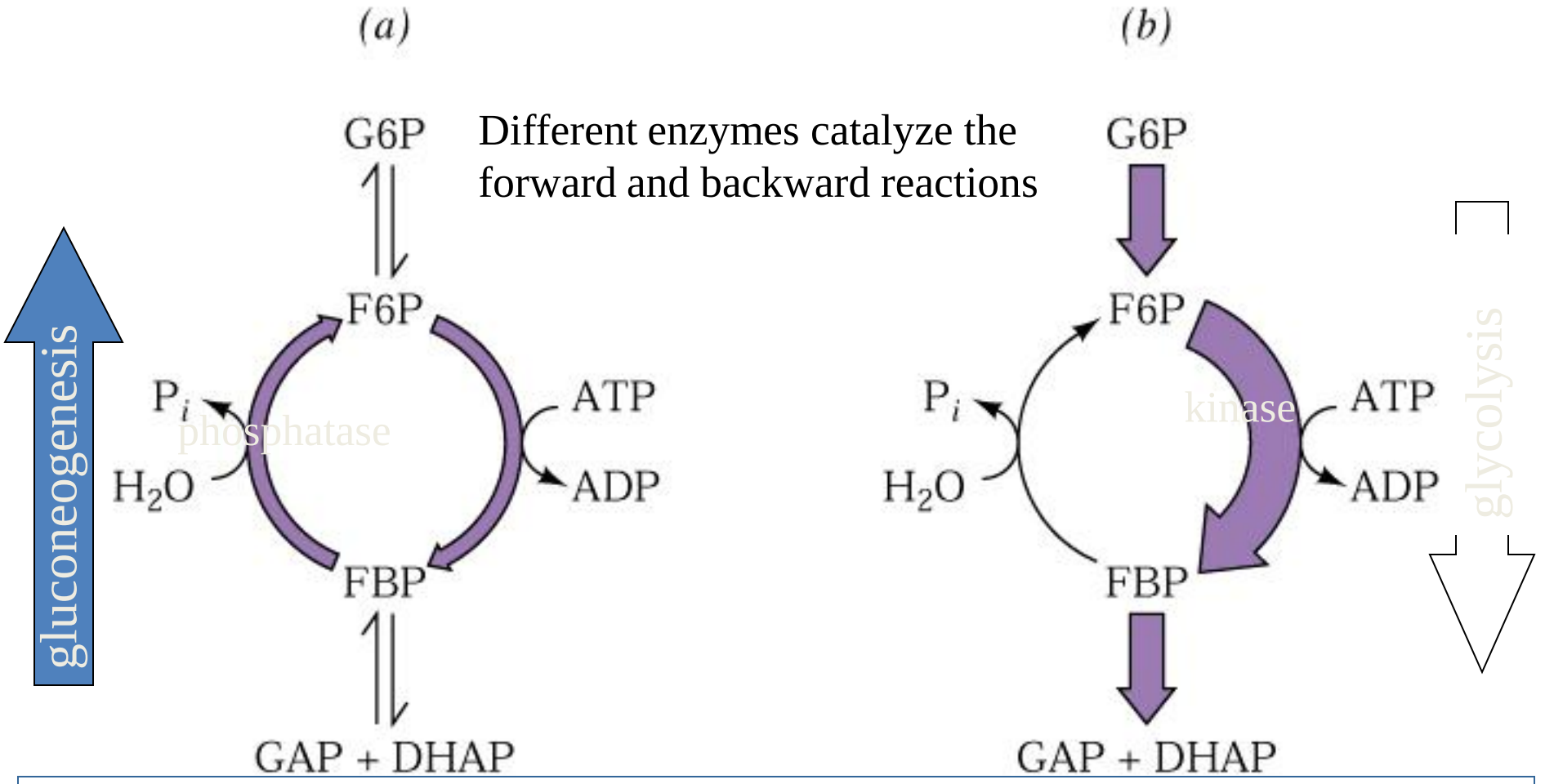


After data from Mansour, T.E. and Ahlfors, C.E., *J. Biol. Chem.* 243, 2523-2533 (1968).

$PFK_T = PFK_R$

Only T state conformation binds ATP at inhibitor site, high ATP, shift to T, low affinity for f6P

Substrate Cycling



Different enzymes catalyze the forward and backward reactions

Reciprocal Regulation: Fructose 2,6 bisphosphate stimulates kinase and inhibits phosphatase

Control of Glycolysis (1)

- Velocity = $\frac{V_{max}[S_t]}{K_m + [S_t]} = \frac{k_{cat}[E_t][S_t]}{K_m + [S_t]}$
- Typical enzyme concentrations, pM-μM
- How can Enzyme levels be controlled?
  - Sequestered storage, triggered release
  - Zymogens (inactive precursors)
    - *quick inefficient*
  - Transcriptional activation (small molecule metabolites or hormones bind to the genes)
    - *slow, efficient*
  - mRNA processing activation; (small molecules bind to untranslated nascent mRNA and affect translation) riboswitches
    - *quick efficient*

When is a ribozyme not just a ribozyme? When it's a riboswitch *http://www.yale.edu/breaker/riboswitch.htm*

RNA Rules Metabolite Production

Small RNA molecules have electrified scientists in recent years with their newly discovered roles in controlling gene expression. The surprises are apparently far from over: Another kind of RNA can detect levels of small molecules that help a cell run smoothly, and it can switch genes on or off depending on the cell's needs.

Molecular biologist Ronald Breaker of Yale University and his colleagues unearthed these multitalented RNA molecules—a class called riboswitches—after wading through decades of scientific literature and puzzling over a handful of unsolved mysteries. Small molecules called metabolites mediate a cell's survival, and metabolite production is fine-tuned by genes that indirectly sense metabolite levels. It had long been assumed that specific proteins bind to a metabolite and trigger expression or repression of genes. But Breaker, as well as researchers at other universities, uncovered seven cases in bacteria, some as old as 30 years, in which frustrated scientists searched in vain for that key protein. There was a reason they couldn't find it, he says: The mystery protein was actually messenger RNA (mRNA). It normally carts the information from DNA to a cell's ribosome, where it's translated into a protein.

Image not available for online use.

Chop shop. A ribozyme (yellow and orange) snips messenger RNA (light blue) into smaller pieces, blocking protein synthesis.

Riboswitches—so named because they are composed of RNA—are portions of specific mRNAs that bind to a metabolite. That changes the shape of the mRNA and switches a gene off, or occasionally on. "It was right in front of you in the literature for 20 or 30 years," says Thomas Tuschl, an RNA researcher at Rockefeller University in New York City. But until Breaker, no one had made the link.

At the AAAS meeting, Breaker reported on his eighth bacterial riboswitch. The switch mediates levels of glucosamine, a key sugar that helps bacteria build their cell walls. Unlike the previous seven (those unsolved mysteries from the past), the new riboswitch is also a ribozyme, a scissorlike molecule that can cut up RNA. It uses this ability to control gene expression. When glucosamine reaches high levels in the cell, the metabolite binds to mRNA and induces the mRNA to cut itself. That prompts a plunge in gene expression, because DNA's message to churn out glucosamine can no longer be transcribed. Mysteriously, the mRNA gets sliced at a site that doesn't code for protein but that still manages to disrupt gene expression. "We don't know why" that happens, says Breaker. "It's a little eerie," notes Sean Eddy, a computational biologist at Washington University in St. Louis, Missouri.

Still, it's clear that "bacteria are loaded" with riboswitches, says Breaker. He's confirmed two more that aren't yet published and has 10 other candidates. The switches have also been found in fungi and plants, and Breaker is planning to start hunting for them soon in animals. —JENNIFER COUZIN

Batteries Powered To Order

As our collection of newfangled electronic gadgets grows, the variety of batteries that power these contraptions keeps expanding as well. But a new generation of smart batteries could put a stop to that. Prototypes have the potential to power a multitude of devices with different energy needs.

Marc Madou, a microengineering expert at the University of California, Irvine, and his colleagues are targeting their efforts at microbatteries, small power cells used to juice devices such as pacemakers, hearing aids, smart cards, and remote sensors. Like all batteries, microbatteries work by shuttling electron-toting compounds from a negatively charged electrode to one that is positively charged, where the electrons are siphoned off. Unlike large batteries, microbattery electrodes are typically made out of thin carbon films stacked on

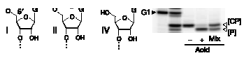
Control of gene expression by a natural metabolite-responsive ribozyme

Wade C. Winkler[1]*, Ali Nahvi[2]*, Adam Roth[1]*, Jennifer A. Collins[1]* & Ronald R. Breaker[1]

[1]*Department of Molecular, Cellular and Developmental Biology, and* [2]*Department of Molecular Biophysics and Biochemistry, Yale University, PO Box 208103, New Haven, Connecticut 06520-8103, USA*

* These authors contributed equally to this work

Most biological catalysts are made of protein; however, eight classes of natural ribozymes have been discovered that catalyse fundamental biochemical reactions. The central functions of ribozymes in modern organisms support the hypothesis that life passed through an 'RNA world' before the emergence of proteins and DNA. We have identified a new class of ribozymes that cleaves the messenger RNA of the *glmS* gene in Gram-positive bacteria. The ribozyme is activated by glucosamine-6-pho (GlcN6P), which is the metabolic product of the GlmS enzyme. Additional data indicate that the ribozyme serves as a meta responsive genetic switch that represses the *glmS* gene in response to rising GlcN6P concentrations. These findings demo that ribozyme switches may have functioned as metabolite sensors in primitive organisms, and further suggest that mode retain some of these ancient genetic control systems.



NATURE |VOL 428 | 18 MARCH 2004 |
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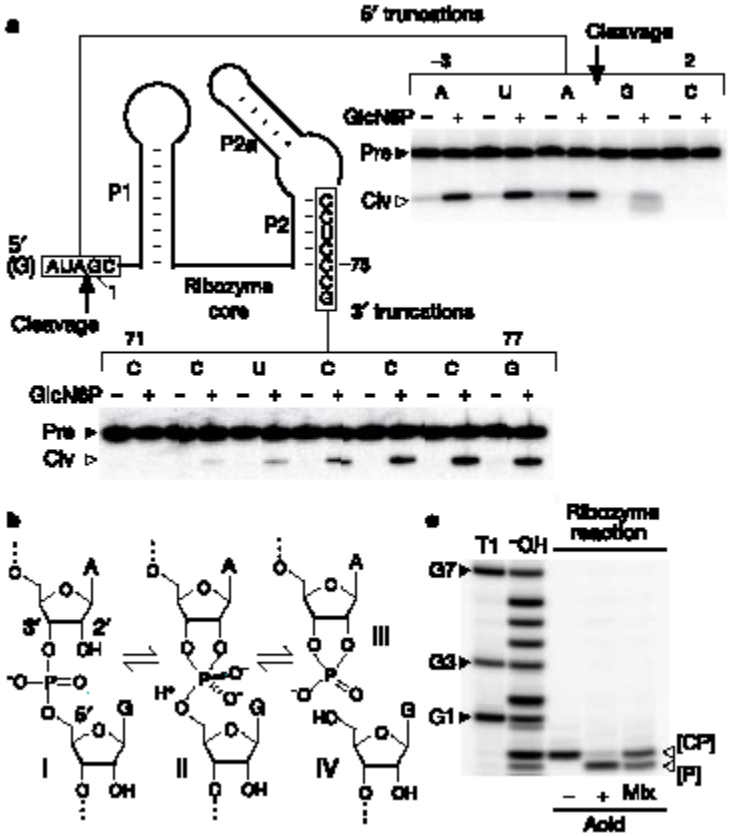

Control of Glycolysis (2)

- Velocity = $\frac{V_{max}[S_t]}{K_m + [S_t]} = \frac{k_{cat}[E_t][S_t]}{K_m + [S_t]}$
- Typical substrate concentrations, 10μM-10 mM
- How can substrate levels be controlled?
  - Sequestered storage (glycogen stores in muscle/liver), hormone triggered release (glucagon)
  - Conversion of related molecule (lactate to pyruvate)
  - Hunger signal to organism (hormone)

Control of Glycolysis (3)

- Velocity = $\frac{V_{max}[S_t]}{K_m + [S_t]} = \frac{k_{cat}[E_t][S_t]}{K_m + [S_t]}$
- Typical k_{cat}, 10^2-10^6 s^{-1}
- How can k_{cat} be increased/decreased?
  - Allosteric effectors (example PFK-gly3)
  - Reversible covalent modification
    - Phosphoryllation, adenylation, methylation, acetyllation, others (example pyruvate dehydrogenase)

Control of Glycolysis (3b)

- Velocity = $\frac{V_{max}[S_t]}{K_m + [S_t]} = \frac{k_{cat}[E_t][S_t]}{K_m + [S_t]}$
- Typical K_m, 10-1000 uM
- How can K_m be increased/decreased?
  - Self Inhibition