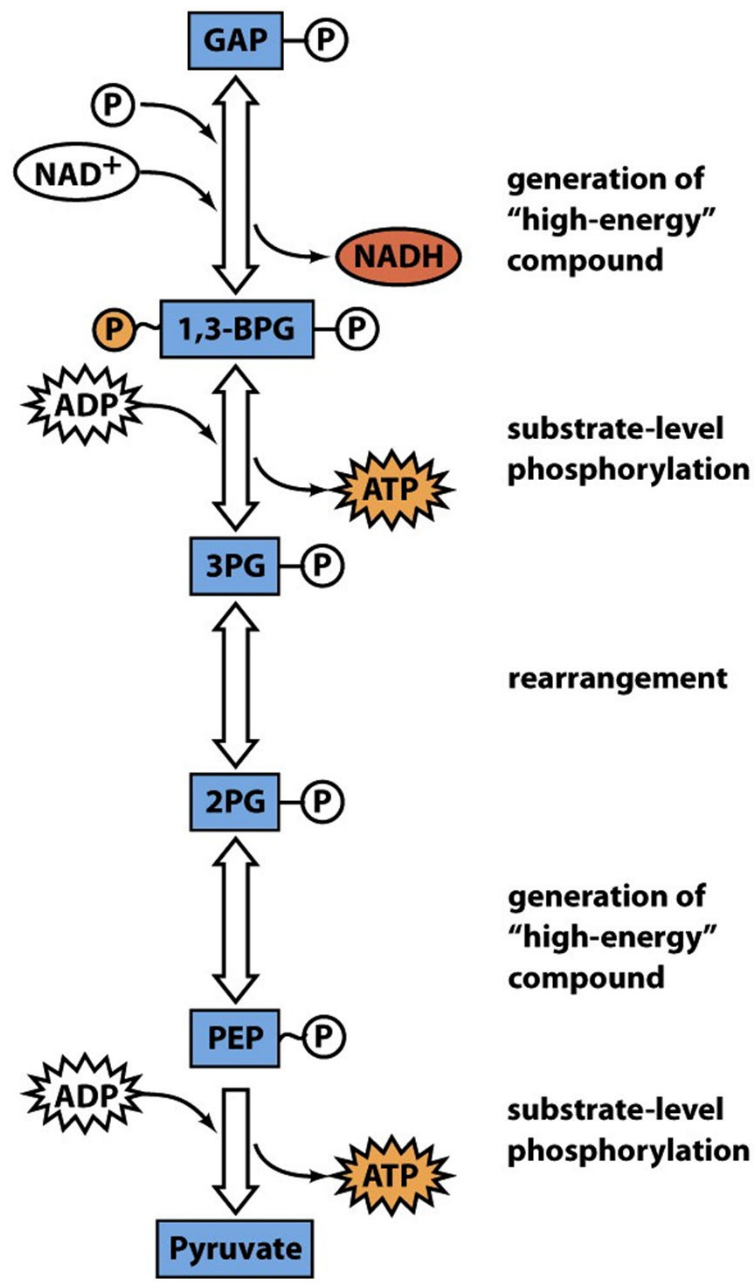
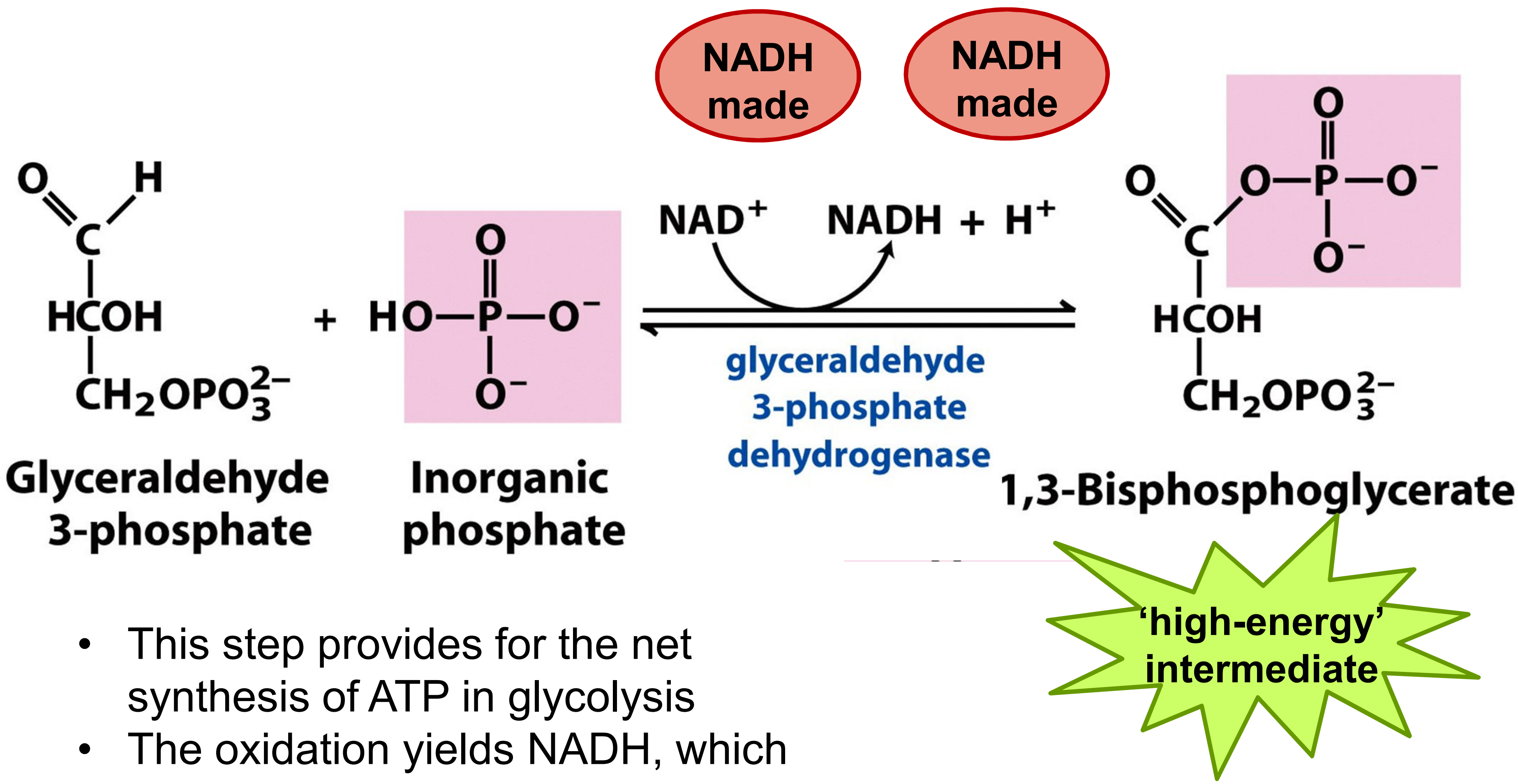
The payoff phase converts 2 GAP to 2 pyruvate, yielding 4 ATP and 2 NADH

$$2GAP + 4ADP + 2P_i + 2NAD^+ \downarrow 2 \text{ pyruvate} + 4ATP + 2H_2O + 2NADH$$



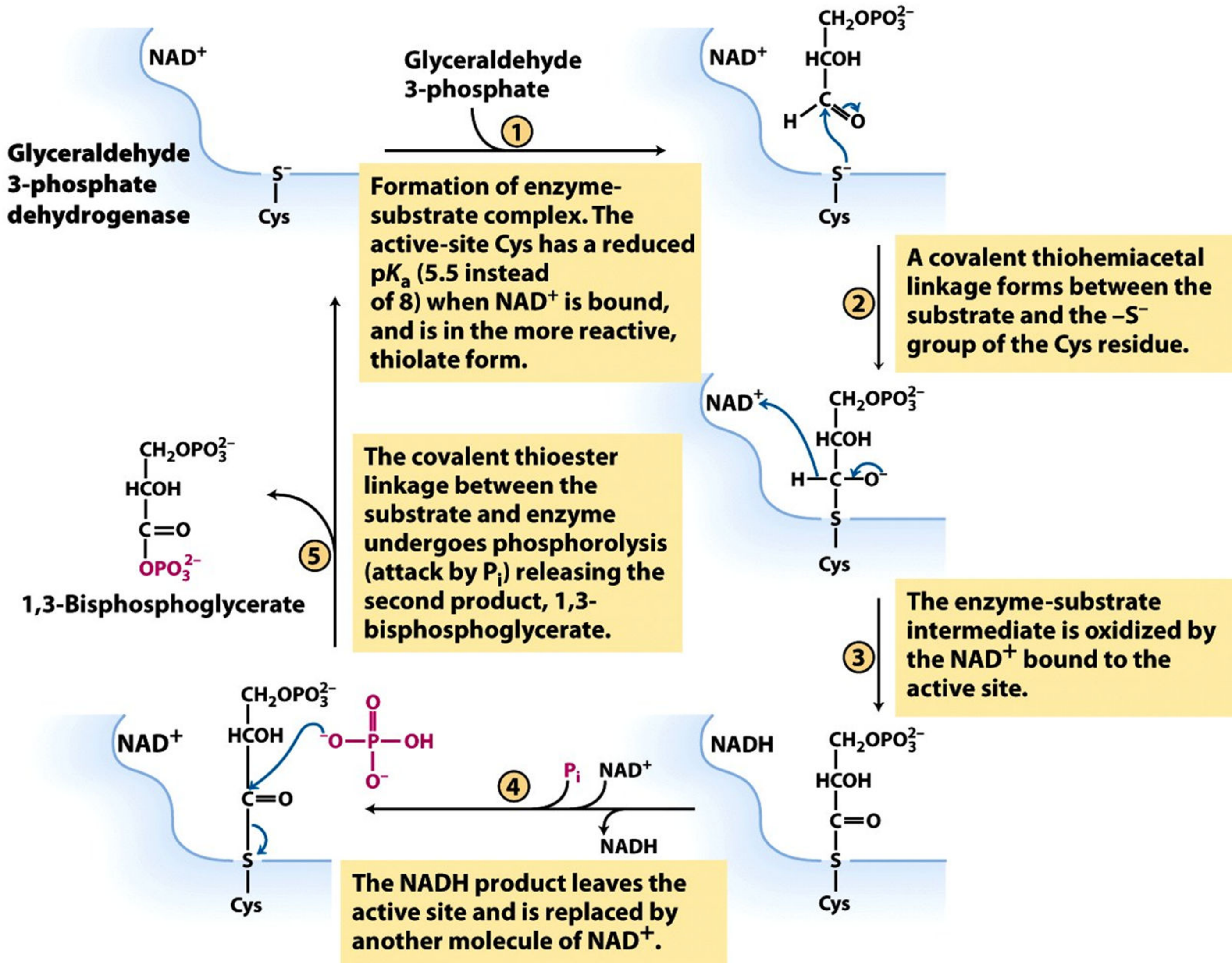
Step 6: GAPDH catalyzes the oxidation and phosphorylation of GAP to 1,3-BPG



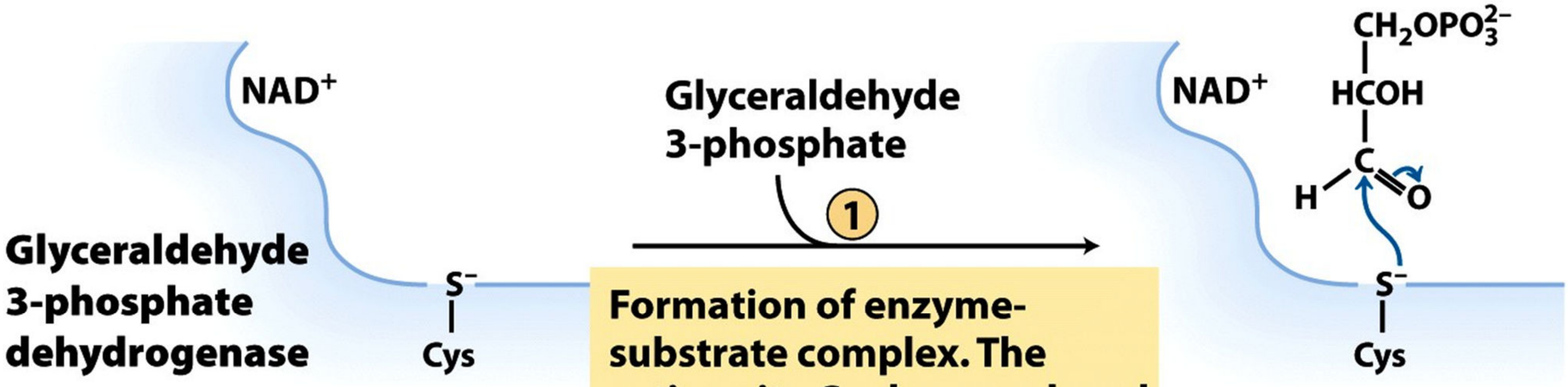
- This step provides for the net synthesis of ATP in glycolysis
- The oxidation yields NADH, which can be used to make more ATP via aerobic metabolism

$\Delta G'^{\circ} = 6.3$ kJ/mol

GAPDH links oxidation to phosphorylation through temporary formation of a thioester



NAD+ binding to GAPDH promotes deprotonation of active-site Cys

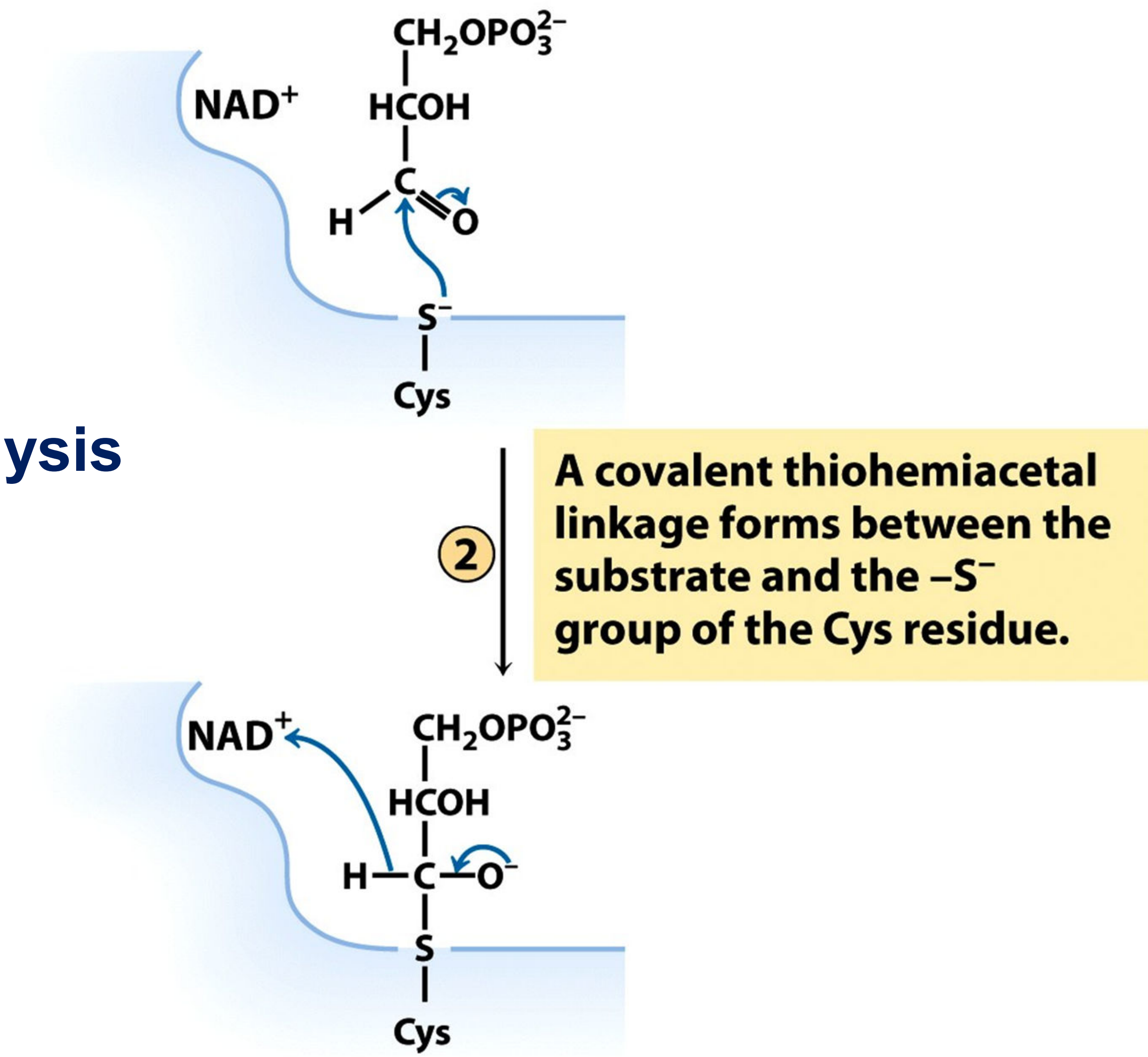


Formation of enzyme-substrate complex. The active-site Cys has a reduced pK_a (5.5 instead of 8) when NAD^+ is bound, and is in the more reactive, thiolate form.

Electrostatic catalysis

Deprotonated Cys is nucleophilic and attacks the electrophilic carbonyl carbon

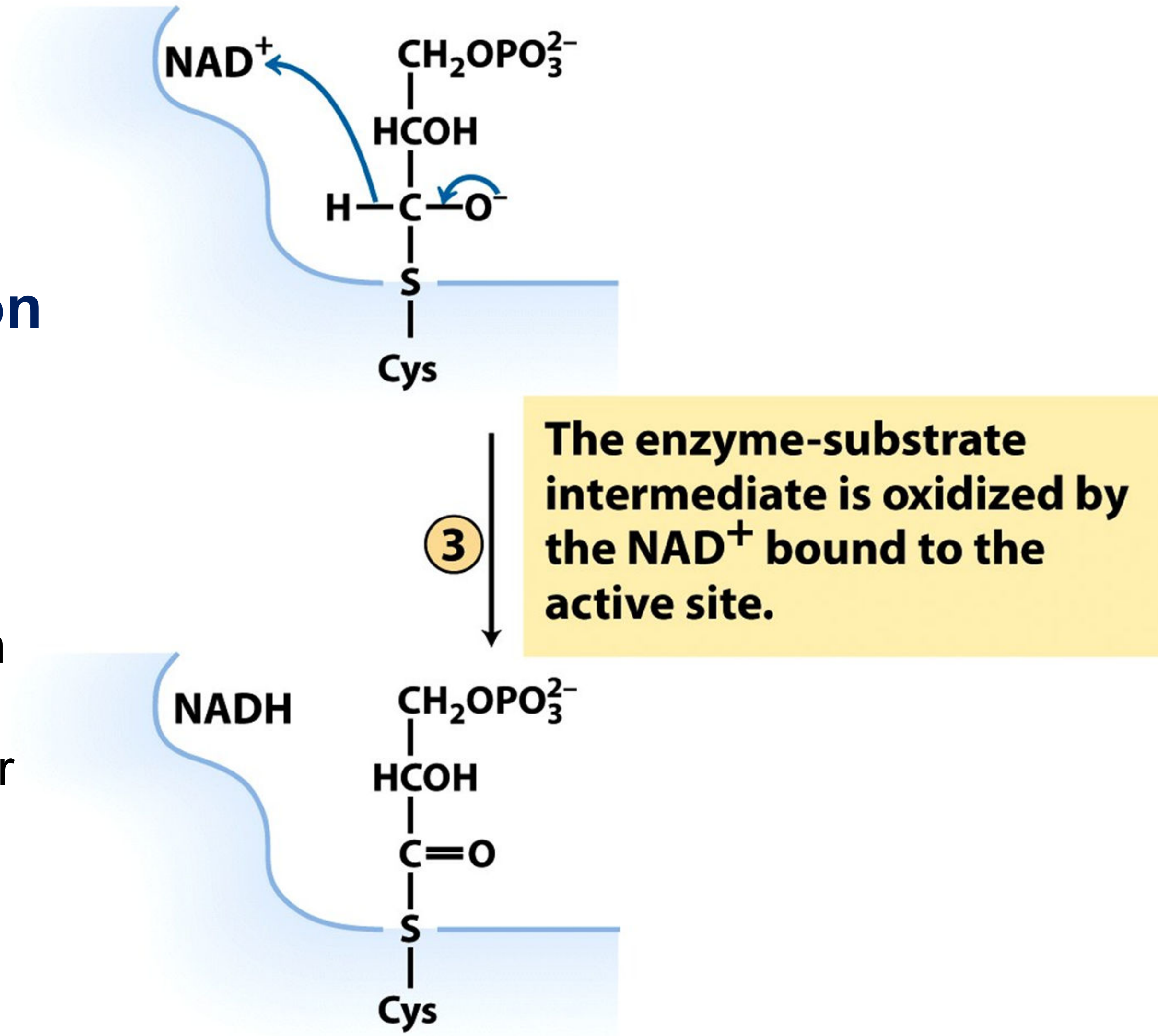
Covalent catalysis



The tetrahedral intermediate kicks out a hydride ion, which is accepted by NAD^+

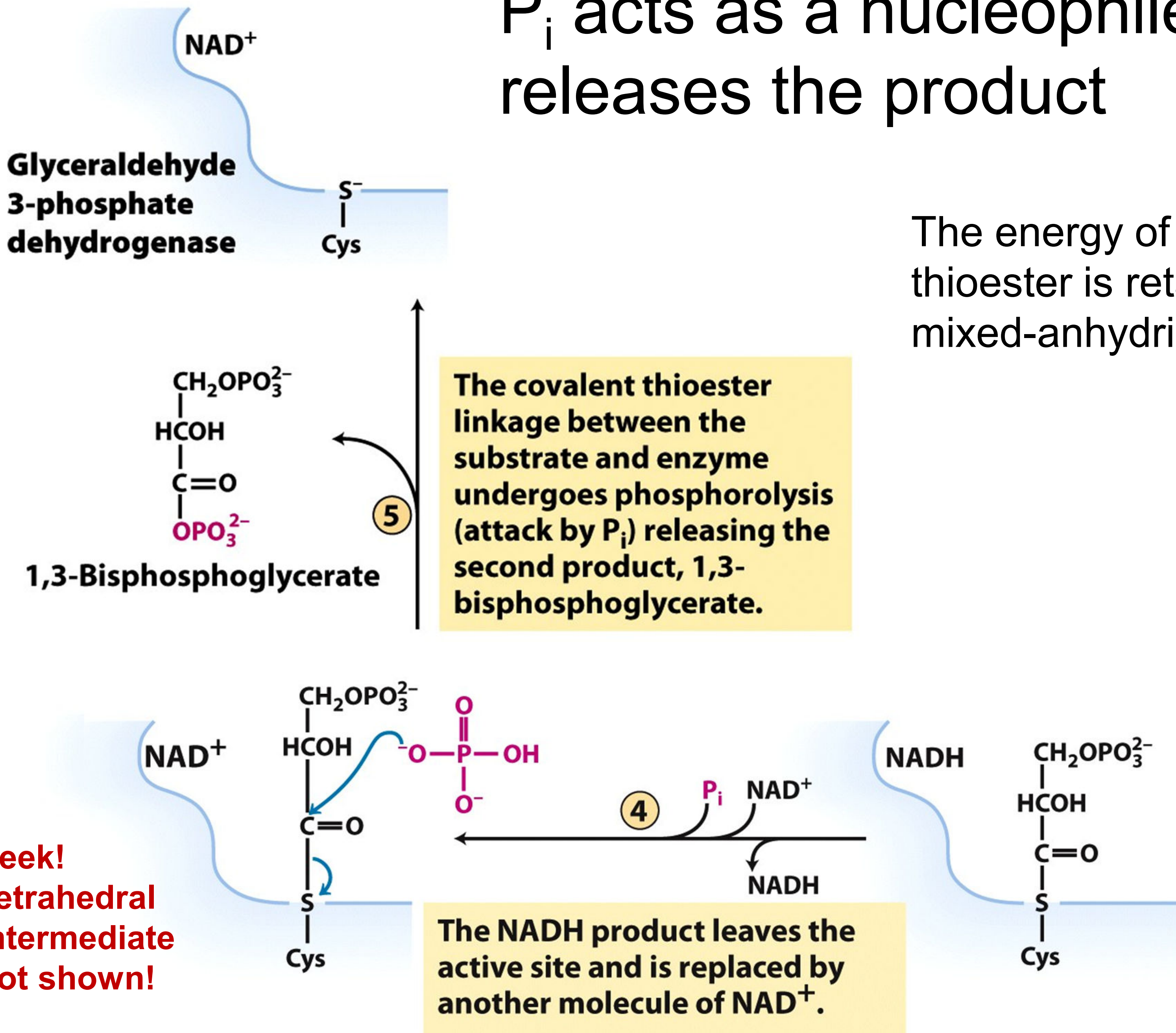
Oxidation-reduction step

The energy that would be released on oxidation is retained through the formation of the thioester



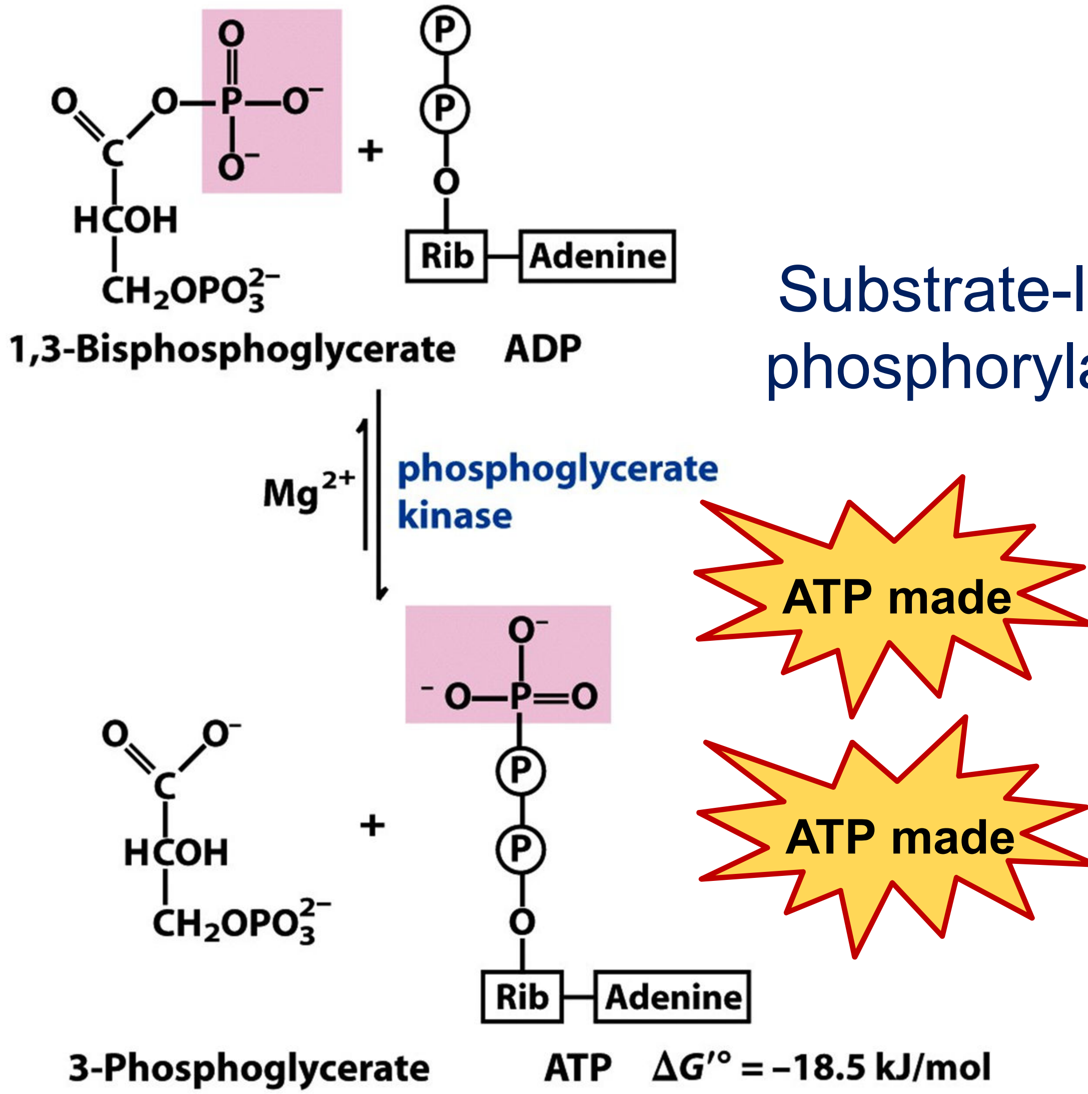
P_i acts as a nucleophile and releases the product

The energy of the broken thioester is retained in the mixed-anhydride product



Eeek! Tetrahedral intermediate not shown!

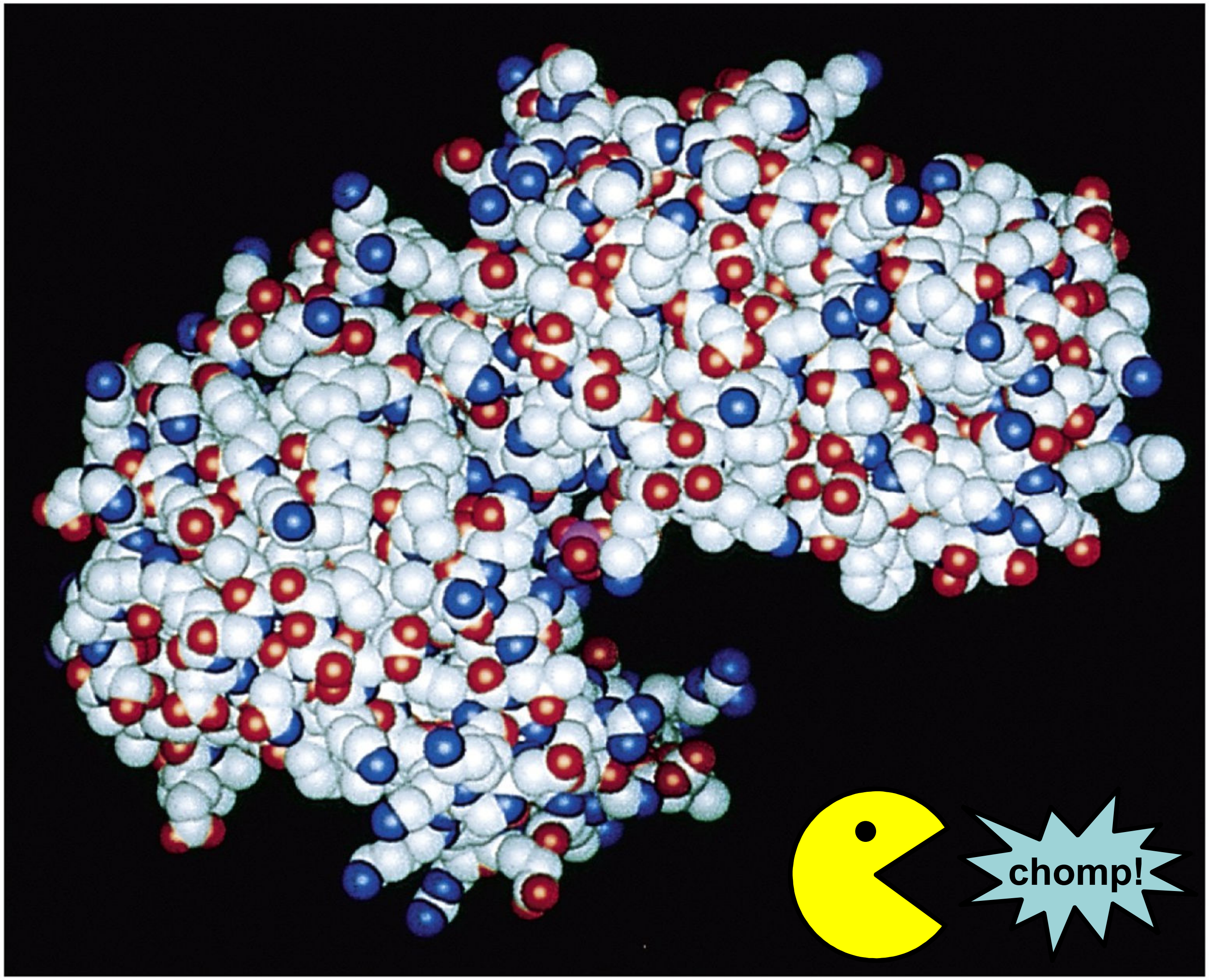
Step 7: Phosphoglycerate kinase (PGK) catalyzes the first synthesis of ATP



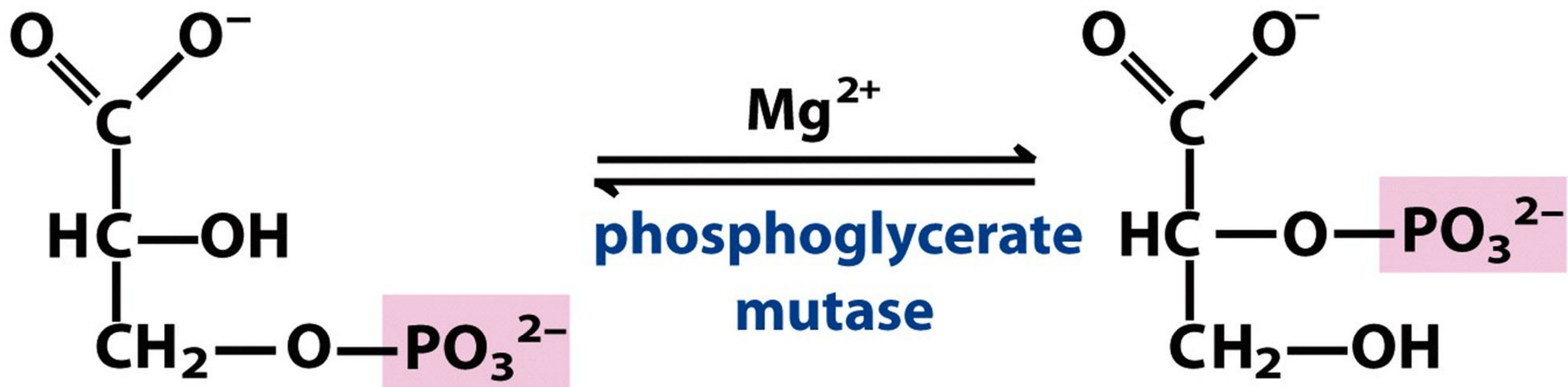
Substrate-level phosphorylation

This step replaces the two ATPs used in the preparatory phase

PGK undergoes a conformational change (induced fit) on binding its substrates



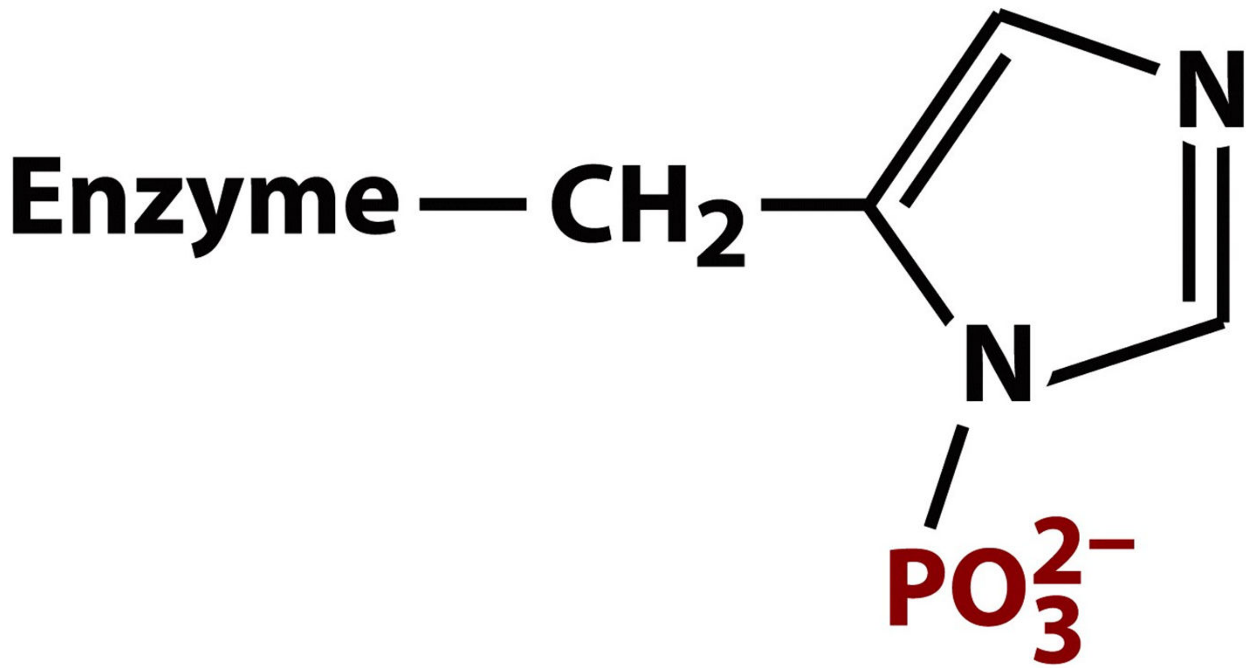
Step 8: PGM catalyzes the isomerization of 3PG to 2PG, in preparation for making PEP



3-Phosphoglycerate 2-Phosphoglycerate

$\Delta G'^{\circ} = 4.4$ kJ/mol

The active PGM enzyme contains a phospho-His in its active site



Phospho-His residue

Phosphoglycerate mutase

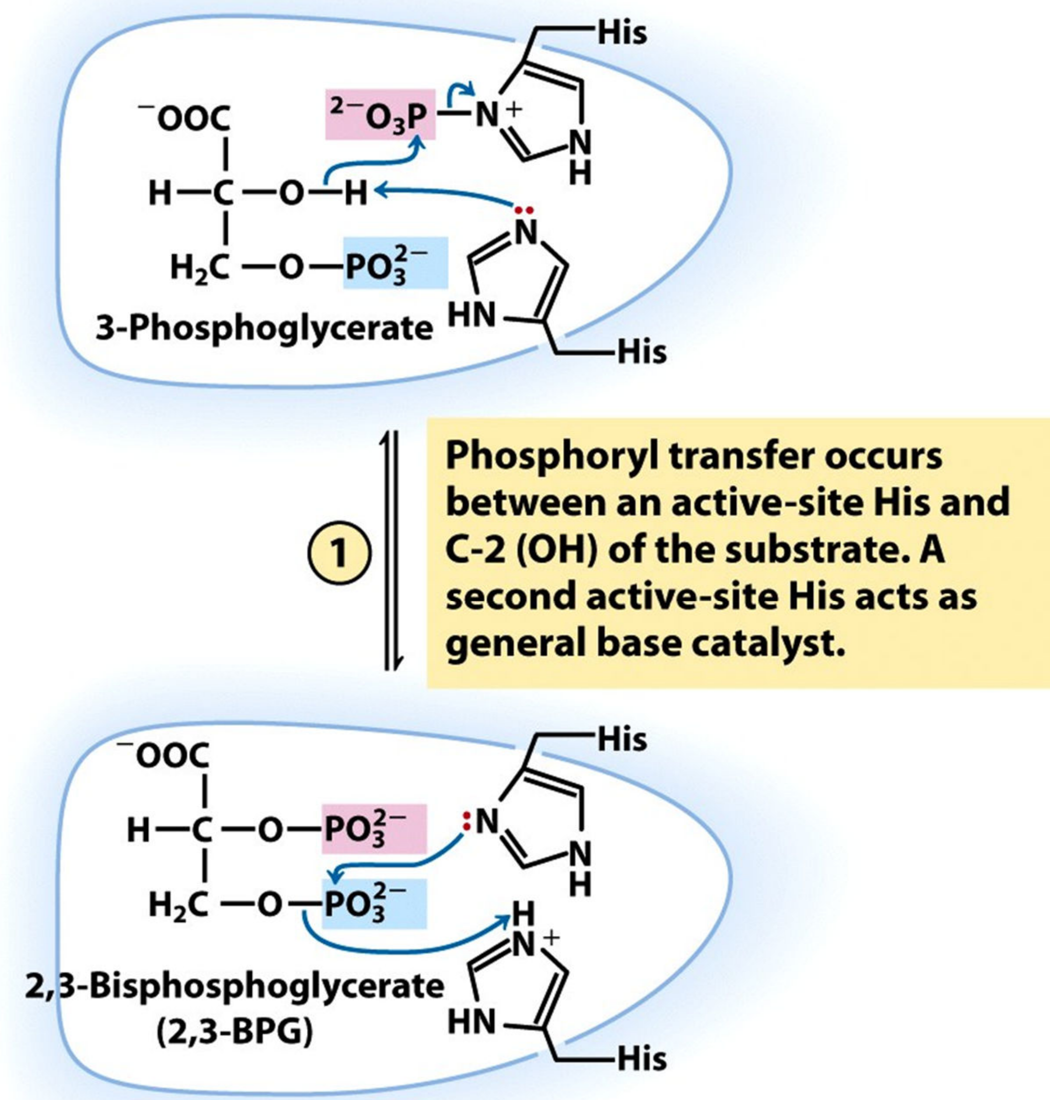


Figure 14-8 part 1
Lehninger Principles of Biochemistry, Fifth Edition

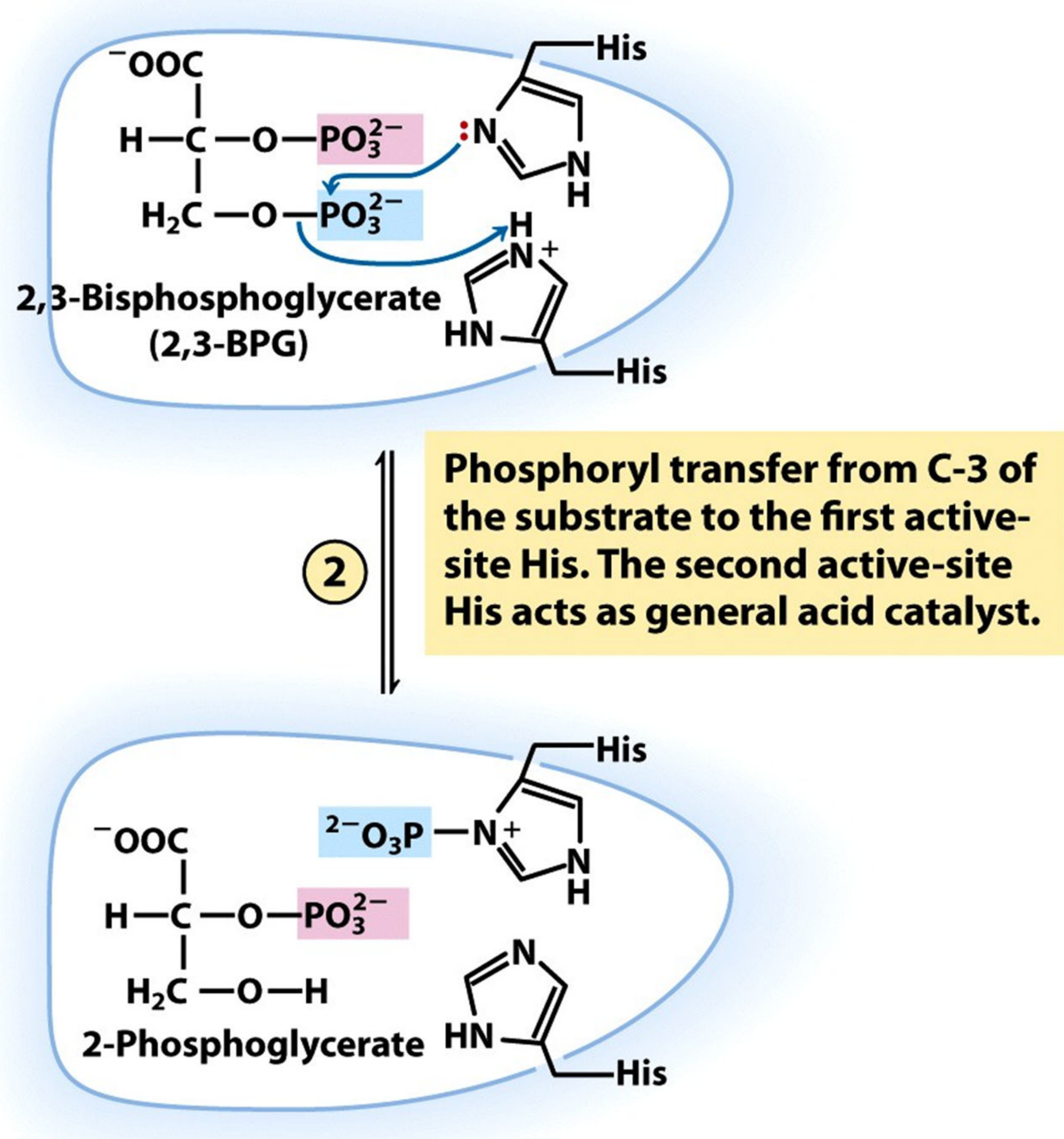
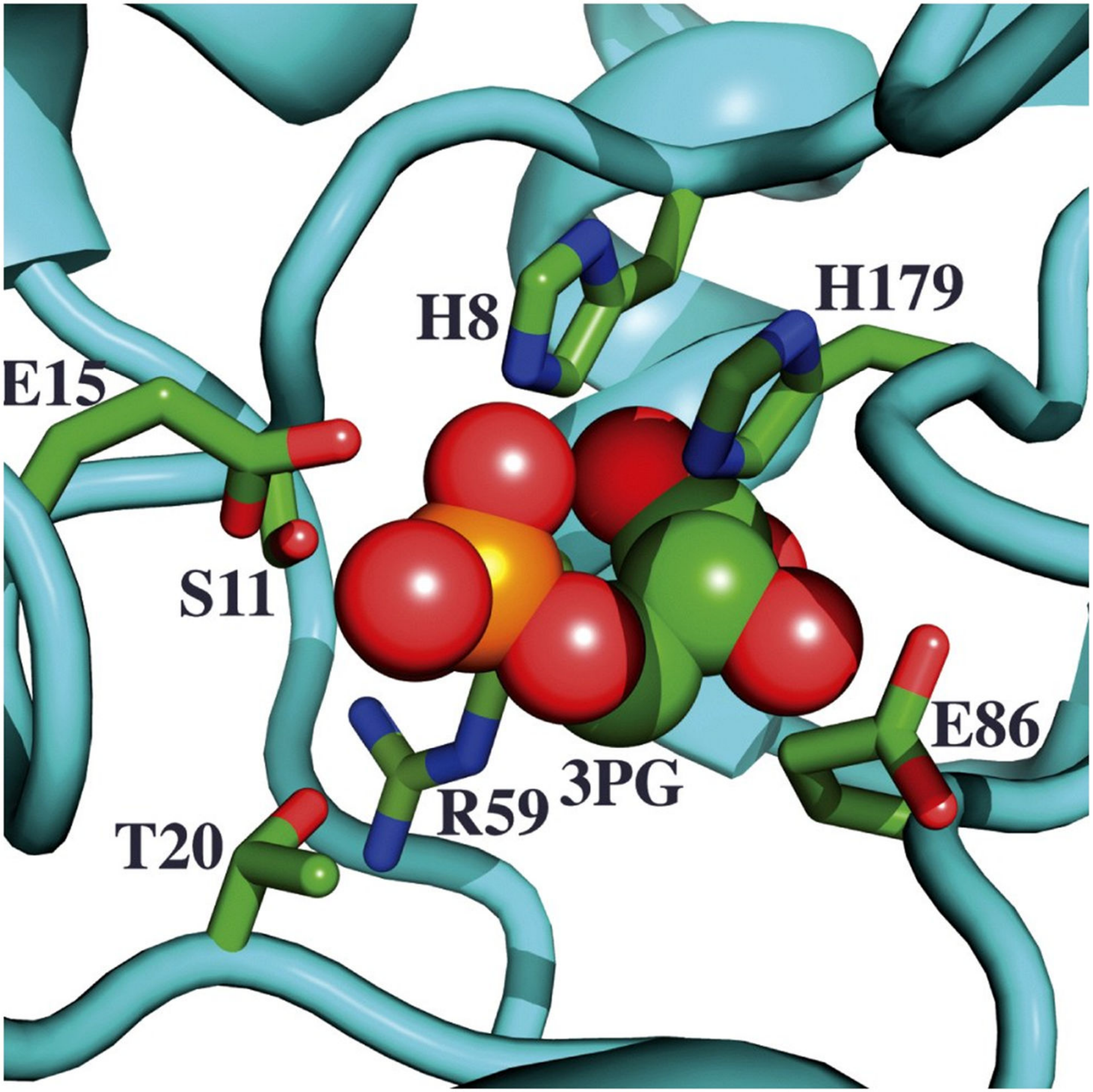
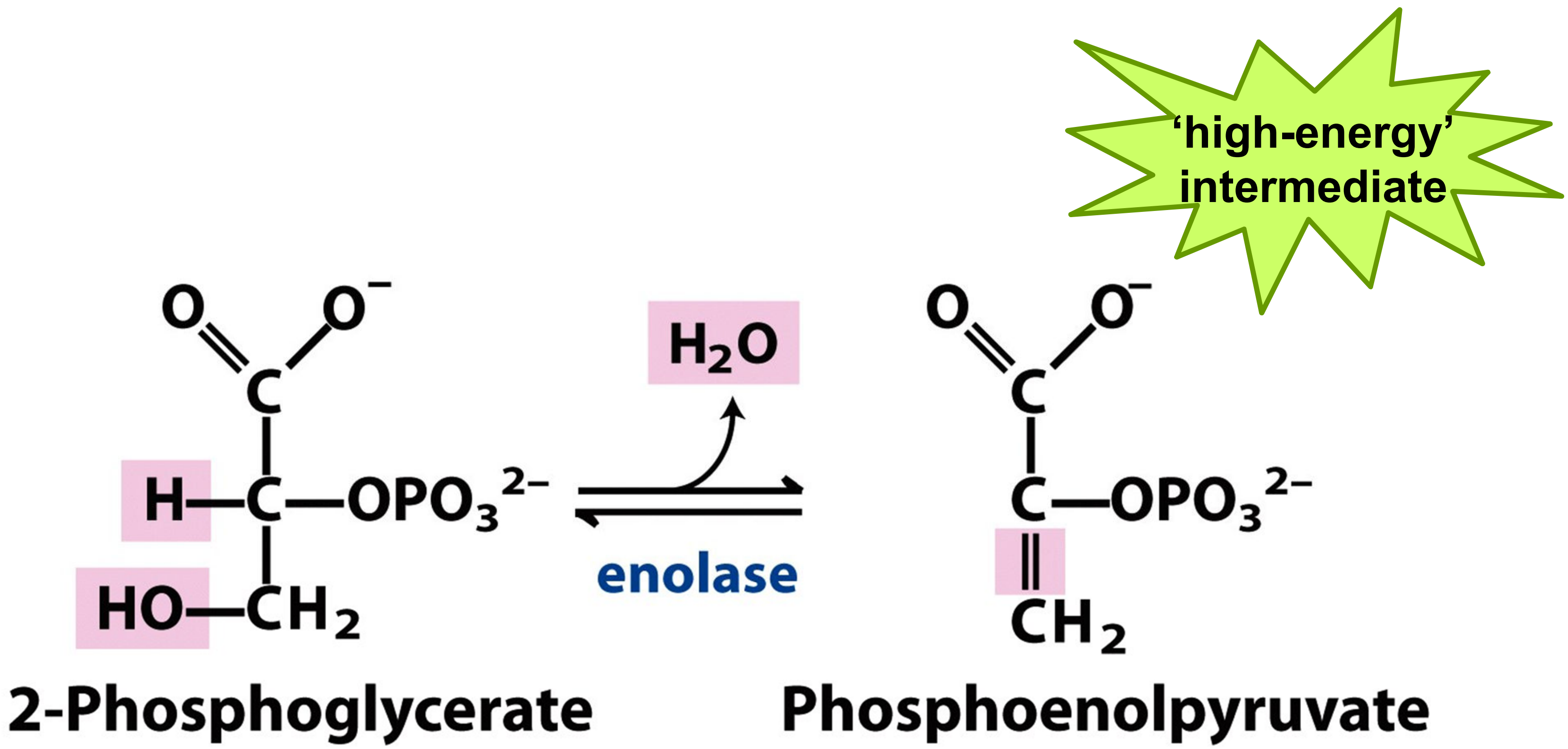


Figure 14-8 part 2
Lehninger Principles of Biochemistry, Fifth Edition

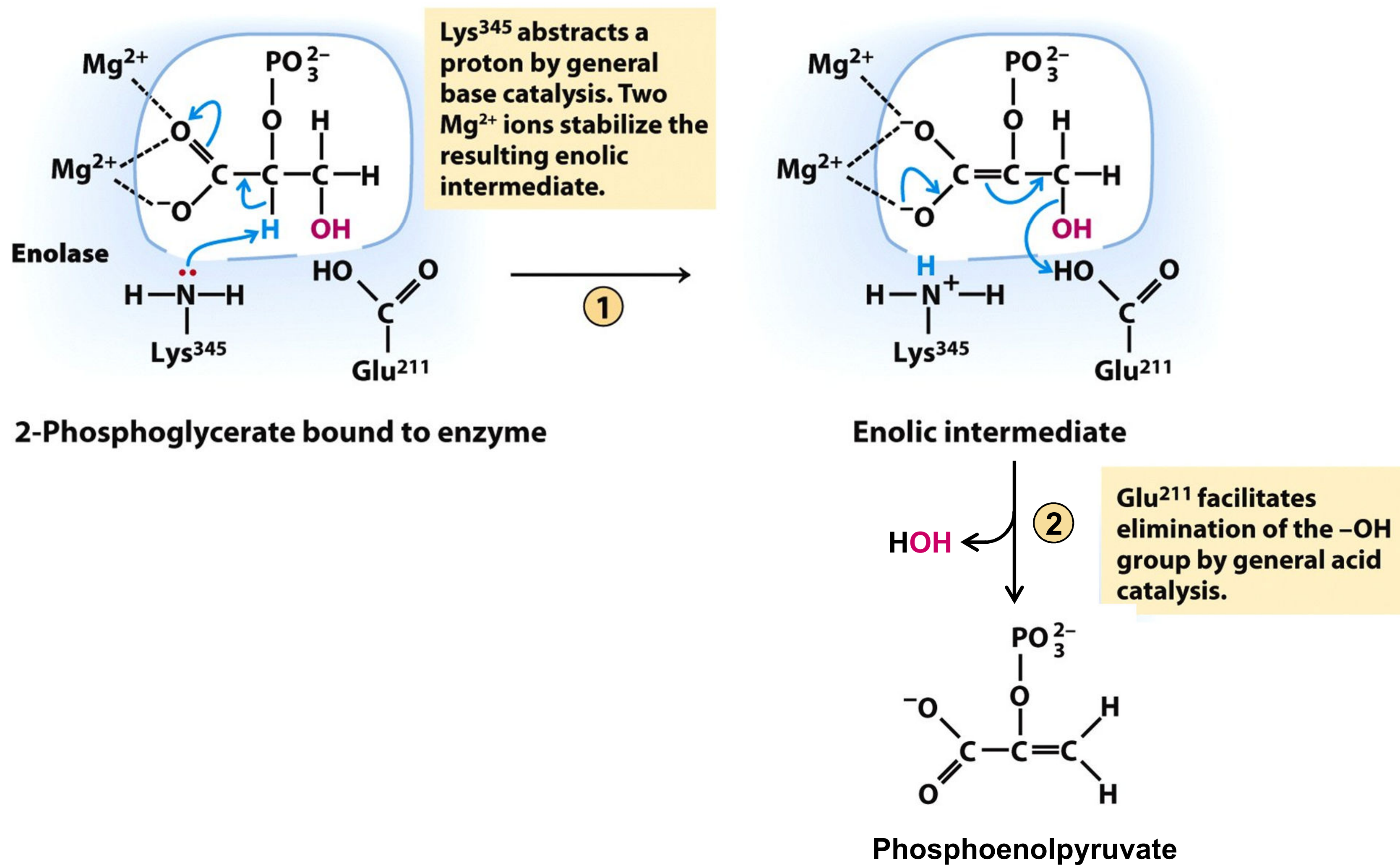

H8
H179
E15
S11
E86
3PG
R59
T20

Step 9: Enolase catalyzes the dehydration of 2PG to PEP, a ‘high-energy’ intermediate



$\Delta G'^{\circ} = 7.5$ kJ/mol

Mg^{2+} ions are essential in the enolate reaction



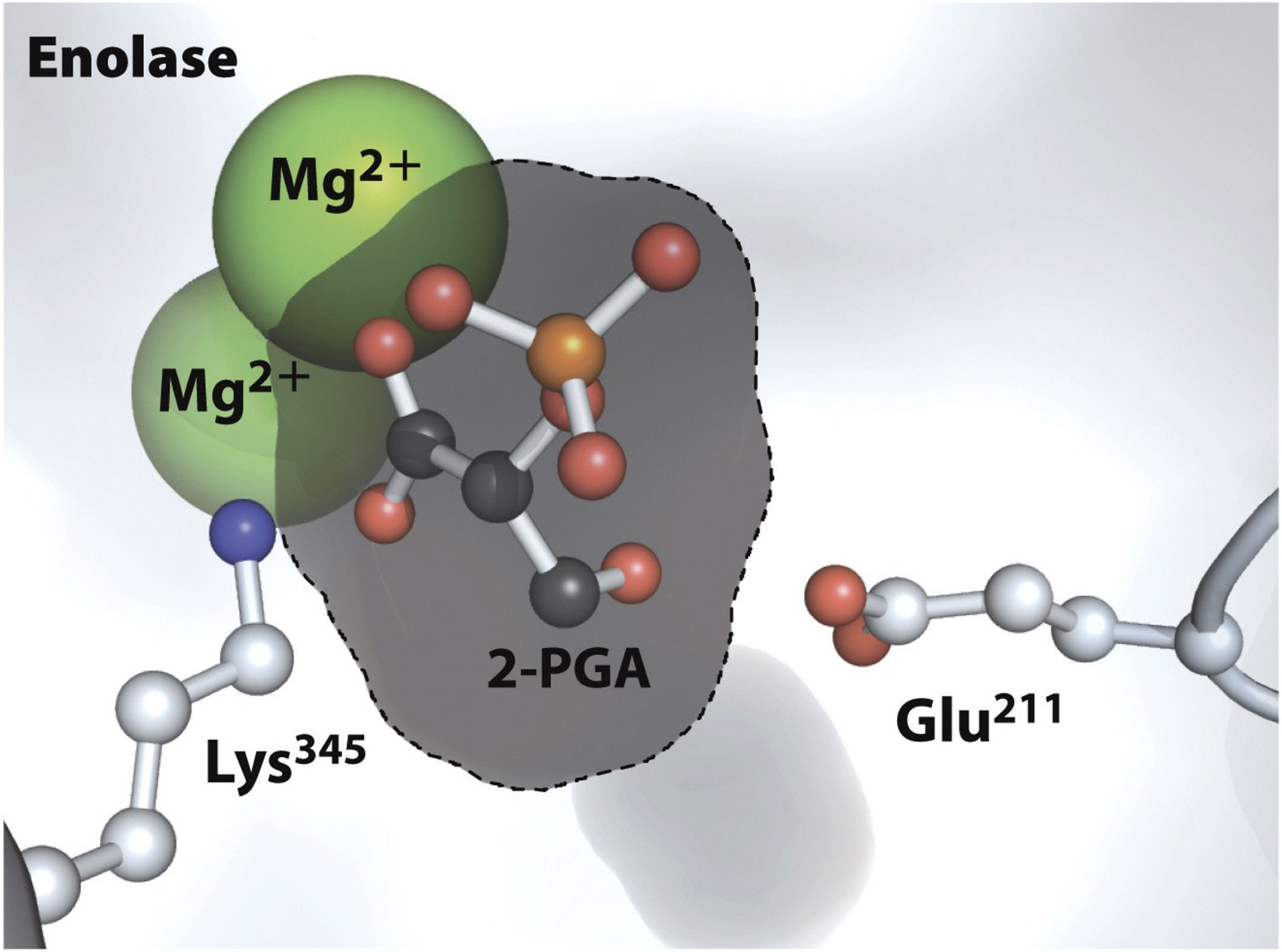
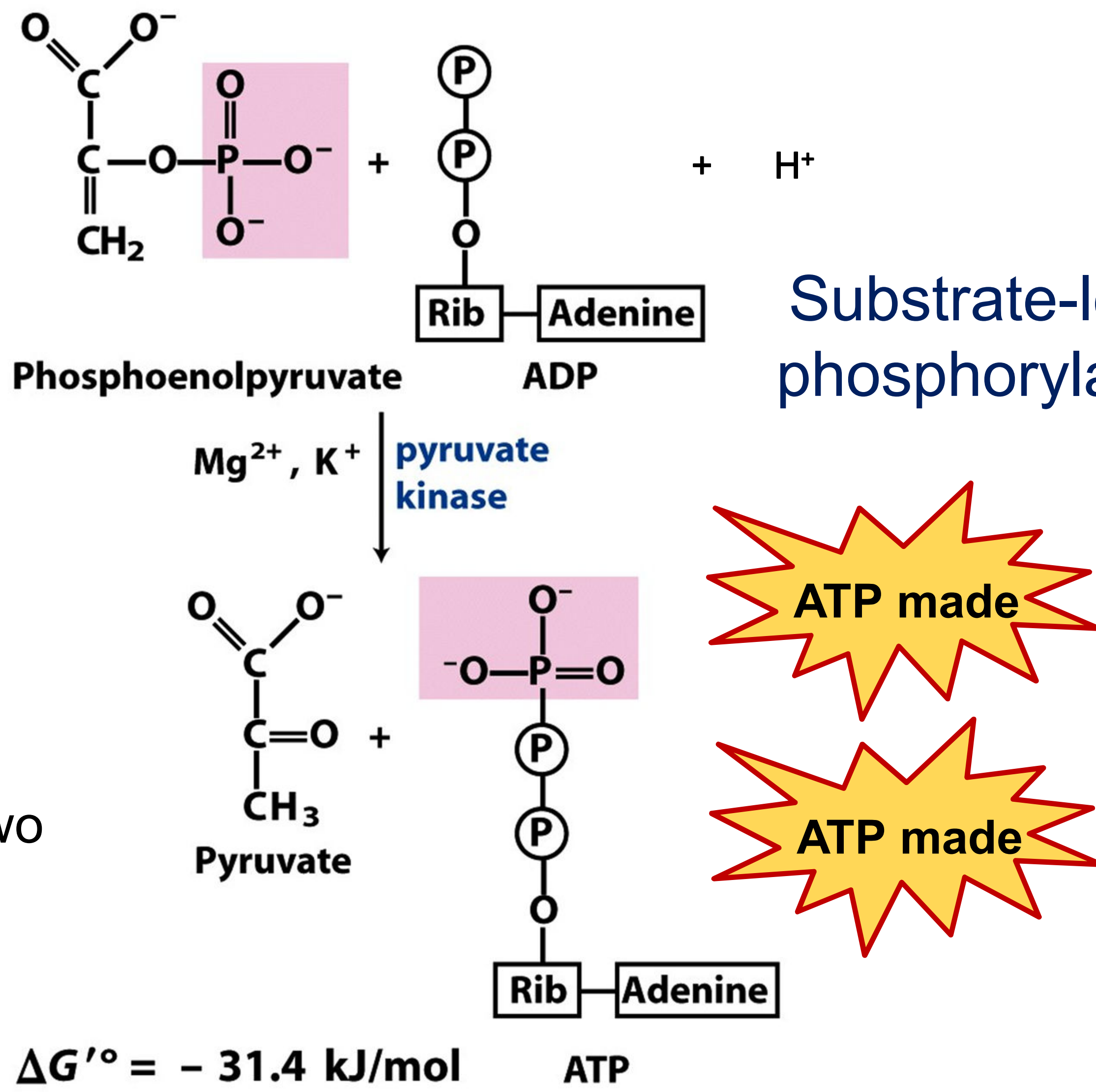


Figure 6-23b
Lehninger Principles of Biochemistry, Fifth Edition

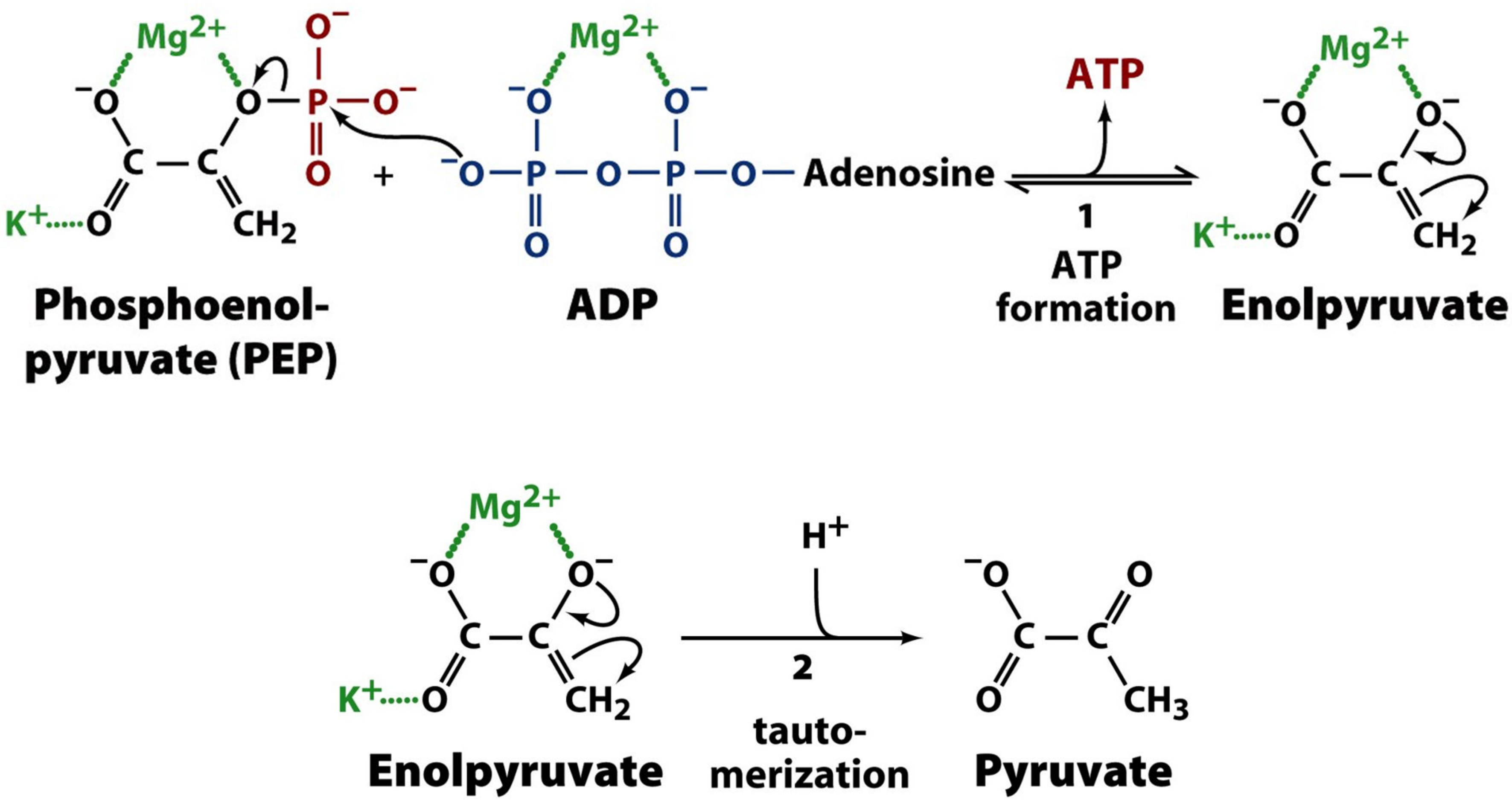
Step 10: Pyruvate kinase catalyzes a phosphoryl transfer from PEP to ADP



Substrate-level phosphorylation

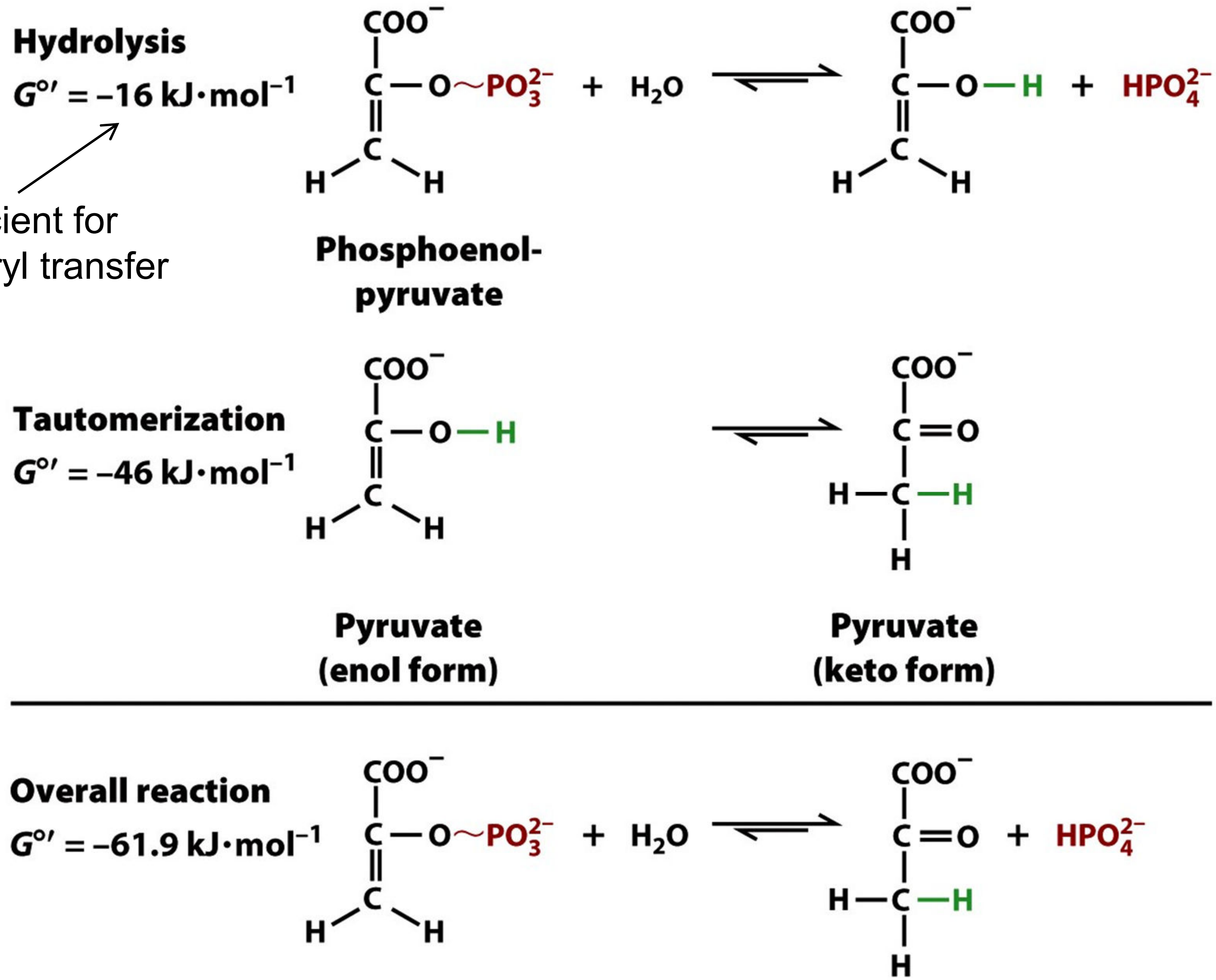
This step yields a net production of two ATPs per glucose

Catalysis by PK depends on Mg^{2+} and K^+ ions



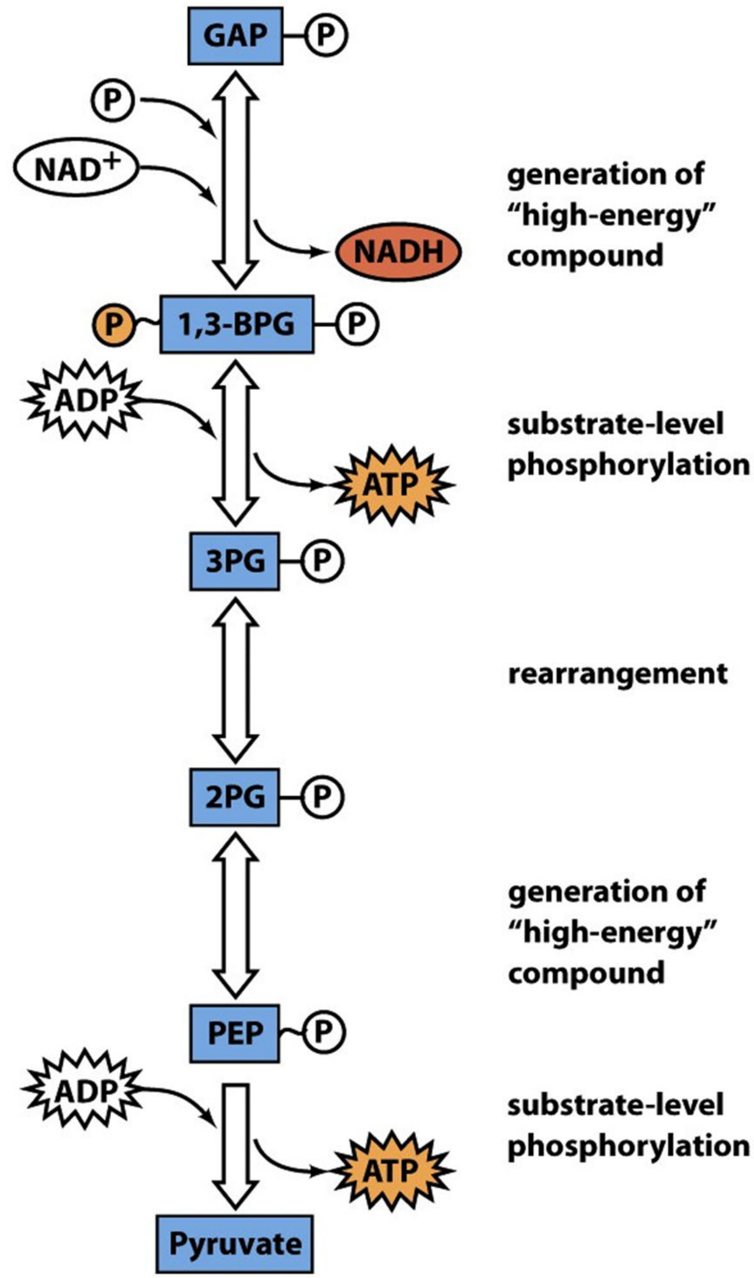
The enol-keto tautomerization of pyruvate is why PEP is a ‘high-energy phosphate’ cpd

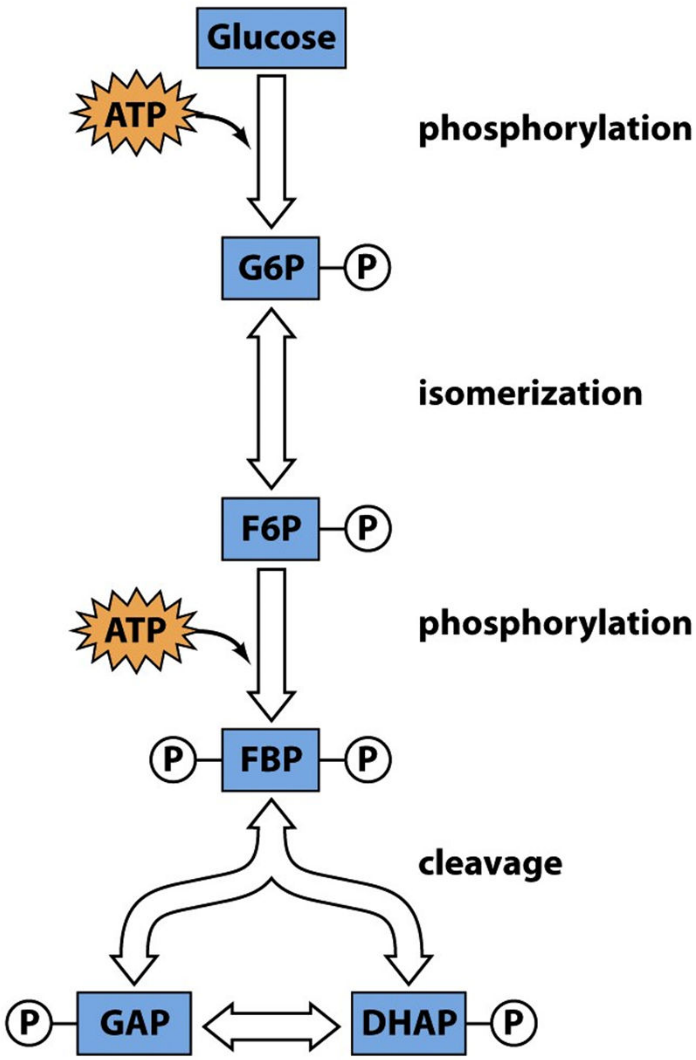
Not sufficient for phosphoryl transfer to ADP

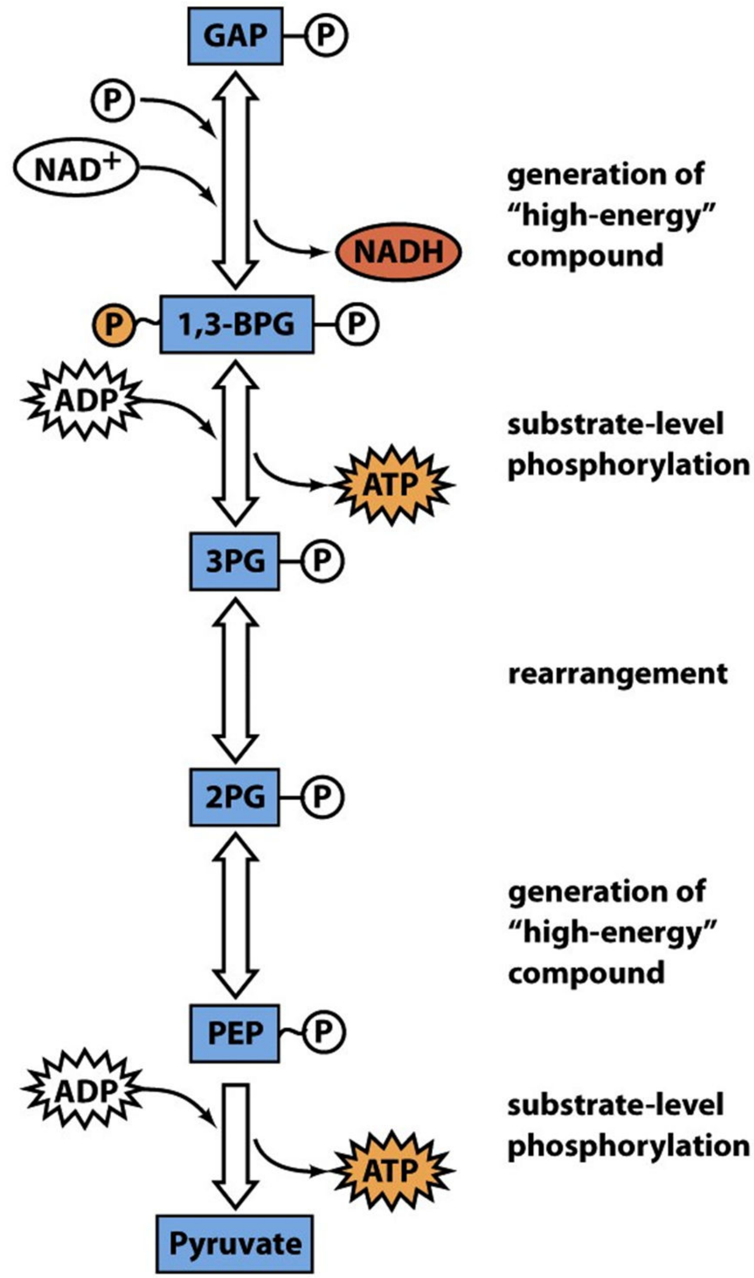


The payoff phase converts 2 GAP to 2 pyruvate, yielding 4 ATP and 2 NADH

$$2GAP + 4ADP + 2P_i + 2NAD^+ \longrightarrow 2\text{ pyruvate} + 4ATP + 2H_2O + 2NADH$$




Glucose
ATP
phosphorylation
G6P
P
isomerization
F6P
P
ATP
phosphorylation
P
FBP
P
cleavage
P
GAP
DHAP
P


GAP
P
P
NAD+
generation of
"high-energy"
compound
NADH
P
1,3-BPG
P
ADP
substrate-level
phosphorylation
ATP
3PG
P
rearrangement
2PG
P
generation of
"high-energy"
compound
PEP
P
ADP
substrate-level
phosphorylation
ATP
Pyruvate