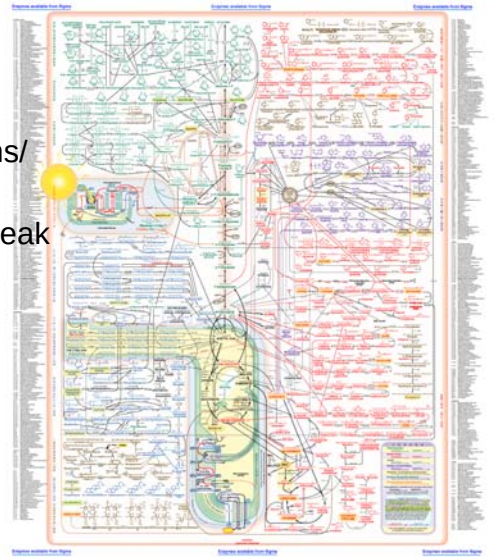
Phosphoryl group transfer

Reactions, mechanisms, and bioenergetics

Four categories of enzymes

1. Group transfer reactions
2. Oxidations/reductions
3. Eliminations/isomerizations/ rearrangements
4. Reactions that make or break C-C bonds



Group transfer reactions

Transfer of an electrophilic group from one nucleophile to another

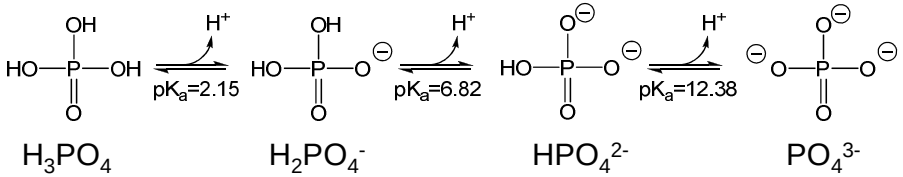
- Acyl group transfer
- Phosphoryl group transfer
- Glycosyl group transfer

Phosphoryl group transfer reactions

- phosphatase
- kinase
- mutase
- phosphodiesterase
- nucleotidyltransferase

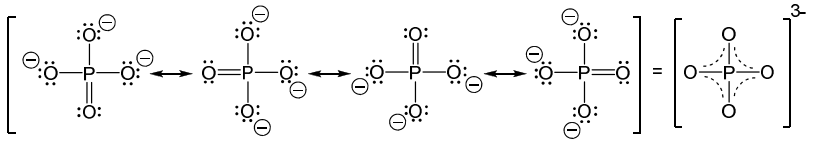
Inorganic phosphate

- Inorganic phosphate:



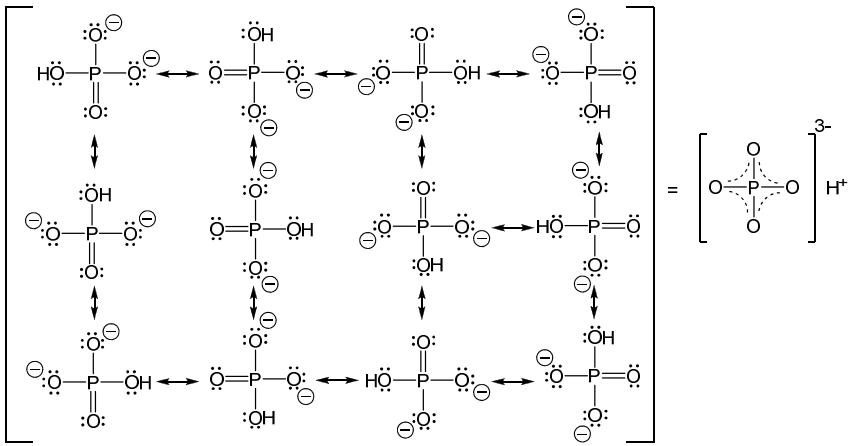
Inorganic phosphate

- Inorganic phosphate resonance forms (fully deprotonated):



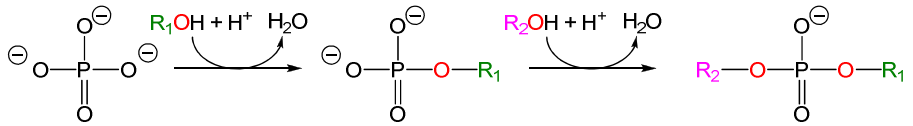
Inorganic phosphate

- Inorganic phosphate resonance forms (monohydrogen phosphate anion):

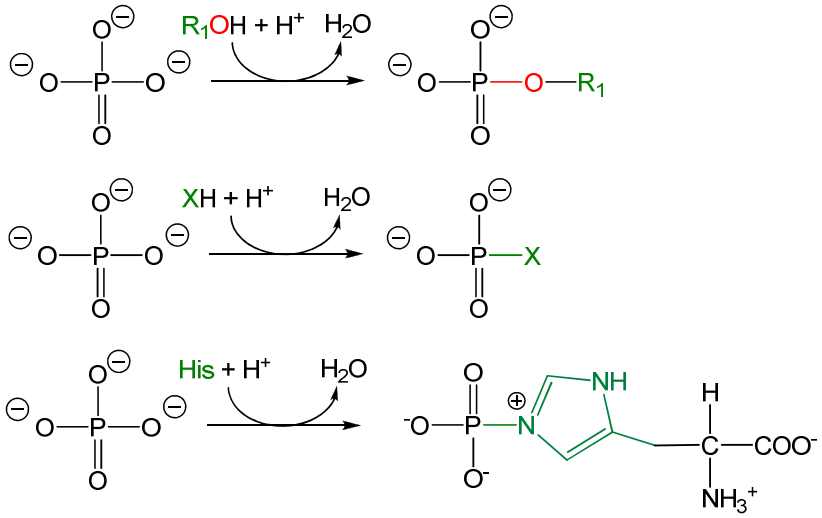


Phosphate esters

- Formed by reaction of phosphate and alcohol
- Shown in deprotonated forms for simplicity
- Double bond only to a nonbonded O
- Position of double bond is arbitrary, remember there are multiple resonance contributors



Phosphate attachment to other atoms than O



Purposes of phosphate linkages

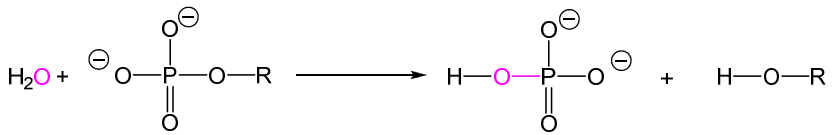
- Convert a poor leaving group to a good one
- Stabilizing structural element
- Increase H_2O solubility of hydrophobic molecules
- Offer reactive handle to biological catalysts
- Enzyme regulation

Phosphoryl group transfer reactions

- phosphatase
- kinase
- mutase
- phosphodiesterase
- nucleotidyltransferase

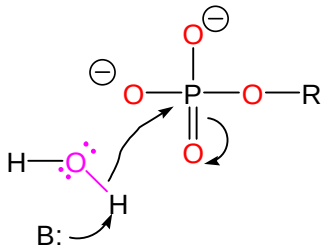
Phosphatase

- phosphoryl group transfer from phosphate monoester to water (hydrolysis of phosphate monoester)
- Products are inorganic phosphate and an alcohol



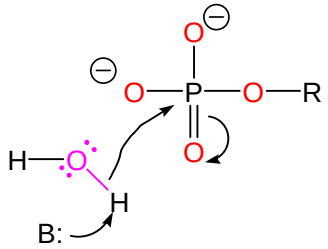
Phosphatase mechanism

- phosphoryl group transfer from phosphate monoester to water
- Mechanism may involve direct nucleophilic attack of hydroxide ion (from water) on electrophilic P (shown below), or may go through a covalent phosphoenzyme intermediate
- A pentavalent intermediate is formed during the reaction



Phosphatase

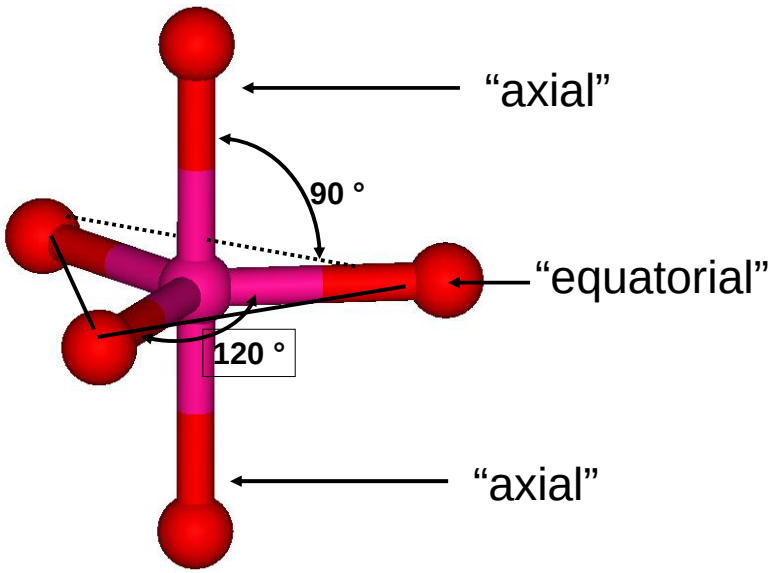
- phosphoryl group transfer from phosphate monoester to water



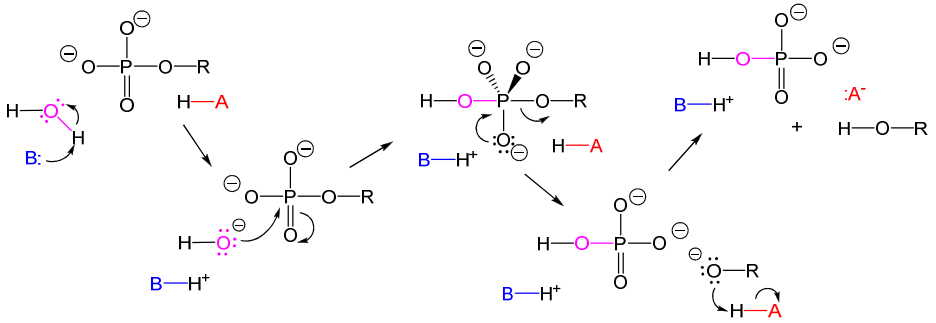
What positions do you think incoming nucleophiles and leaving groups will occupy in the pentavalent intermediate formed during phosphoryl group transfer ?

A. Both equatorial
B. Both axial
C. Incoming axial, leaving equatorial
D. Leaving equatorial, incoming axial

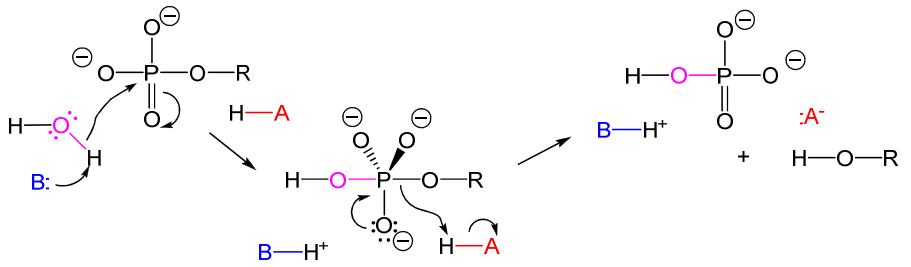
Trigonal bipyramidal electron pair geometry



Phosphatase mechanism with water as the direct nucleophile: "stepwise" involvement of general base and general acid

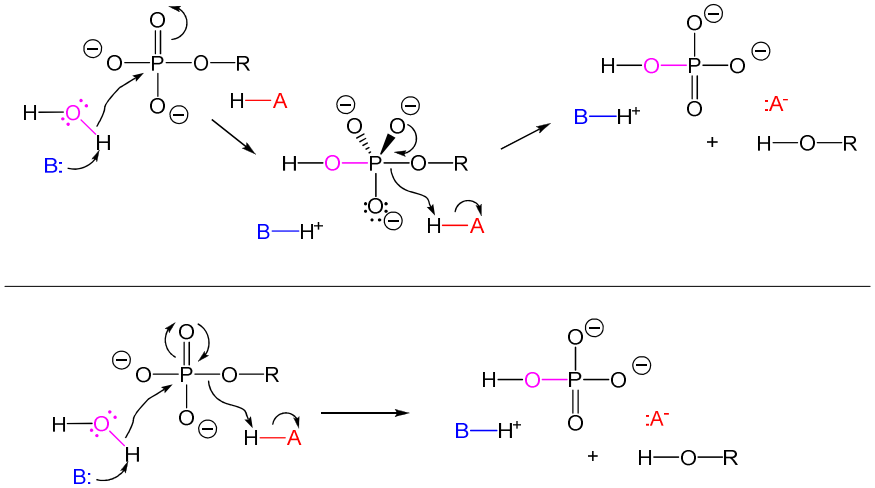


Phosphatase mechanism: “concerted” involvement of general base and general acid

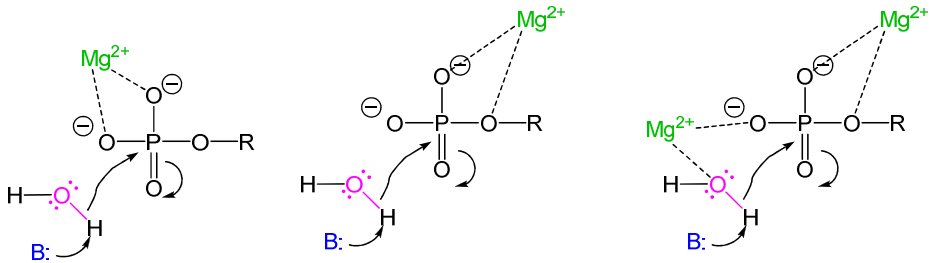


In general, showing the concerted mechanisms for the involvement of general acids and bases is preferred, as species such as the hydroxide and alkoxide ions probably aren’t really formed as discrete species

Phosphatase mechanism: “concerted”, and short-hand abbreviation for forming the pentavalent intermediate



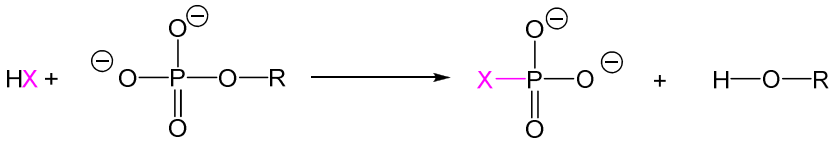
Possible roles for Mg^{2+} in a phosphatase mechanism



In general, divalent metal ion(s) are involved in phosphoryl group transfer reactions

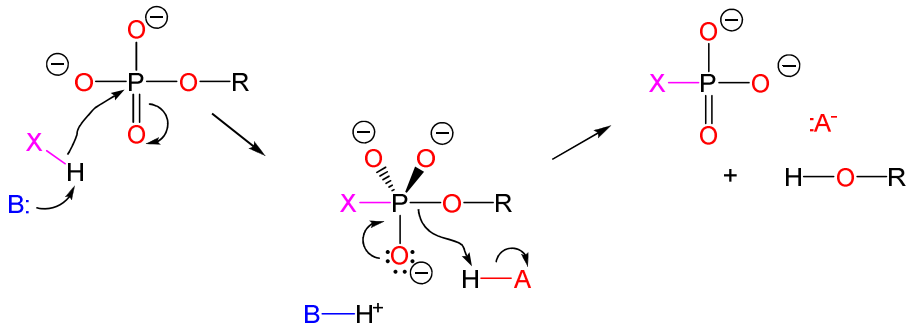
Kinase

- Phosphoryl group transfer from a phosphate monester to an acceptor other than water (i.e. organic acceptor)
- Generally, ATP donates a phosphoryl group, or ADP accepts one
- Nucleophile is usually O but can be something else (e.g. N)



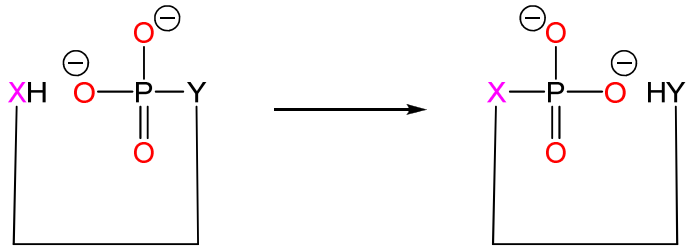
Kinase mechanism

- Same as phosphatase mechanism, but nucleophile is "X-" rather than "OH-"
- General base and acid required as shown



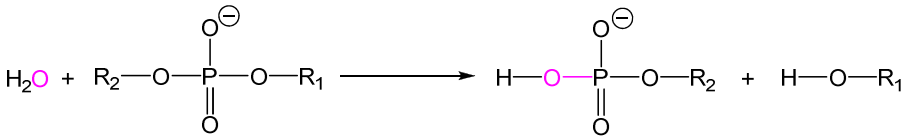
Mutase

- Intramolecular phosphoryl group transfer
- As we will see later, the mechanism does not involve direct phosphoryl transfer between donor and acceptor



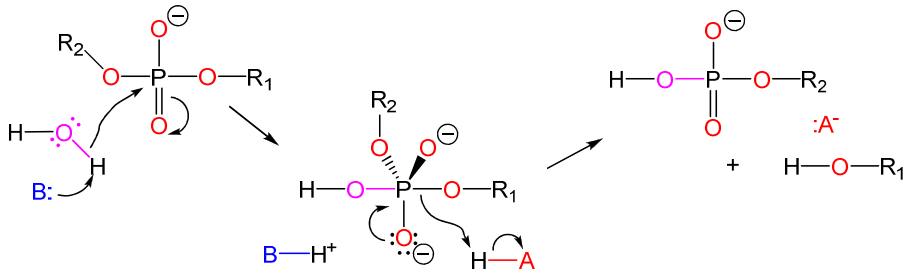
Phosphodiesterase

- Hydrolysis of a phosphodiester
- Products are a phosphate monoester and an alcohol



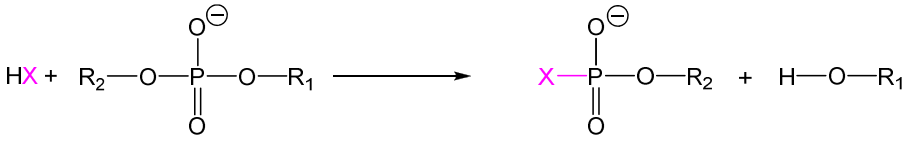
Phosphodiesterase mechanism

- Same as phosphatase mechanism, except a phosphodiester is hydrolyzed rather than a phosphomonoester



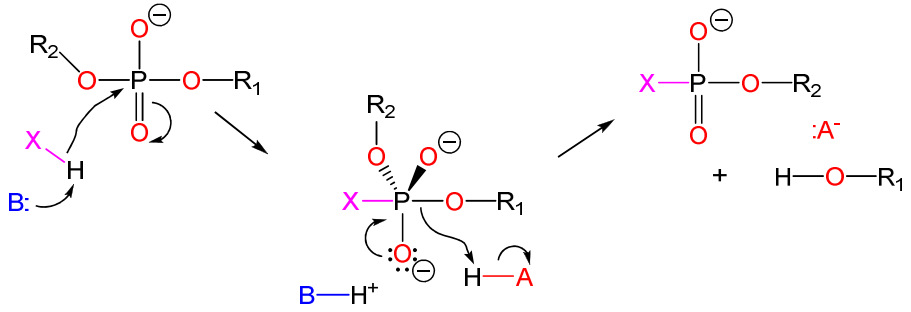
Nucleotidyltransferase

- Phosphotransferases that transfer phosphate monoesters (specifically nucleotides) to organic acceptors
- An example is AMP transfer from ATP to an organic acceptor (adenylylation)



Nucleotidyltransferase mechanism

- Same as kinase mechanism, except a phosphodiester is substrate rather than a phosphomonester



Group transfer reactions

Transfer of an electrophilic group from one nucleophile to another

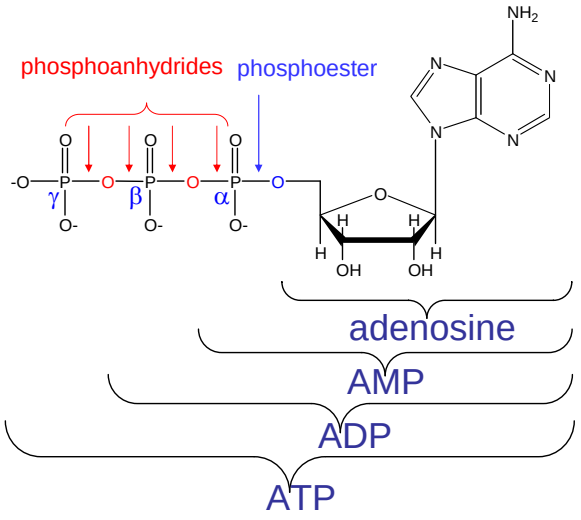
- Acyl group transfer
- Phosphoryl group transfer
  - Phosphatase
  - Mutase
  - Kinase
  - Phosphodiesterase
  - Nucleotidyl transferase
- Glycosyl group transfer

Note similarities in reactions

Thermodynamics of phosphate ester hydrolysis (phosphoryl group transfer to water)

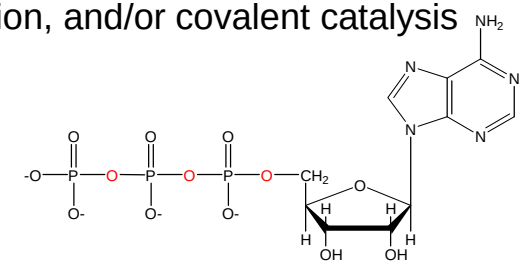
1. Group transfer reactions
   - Phosphoryl group transfer
     - Acceptor of the phosphoryl group is water
2. Oxidations/reductions
3. Eliminations/isomerizations/ rearrangements
4. Reactions that make or break C-C bonds

Adenosine triphosphate



ATP hydrolysis

- Initiated by attack of hydroxide on one of the three electrophilic phosphorus atoms
- In theory, any of the phosphate ester bonds can be hydrolyzed
- Enzymatically, ATP hydrolysis to ADP and P_i, or AMP and PP_i, is most common
- Enzymatic hydrolysis can be assisted by general base metal ion, and/or covalent catalysis



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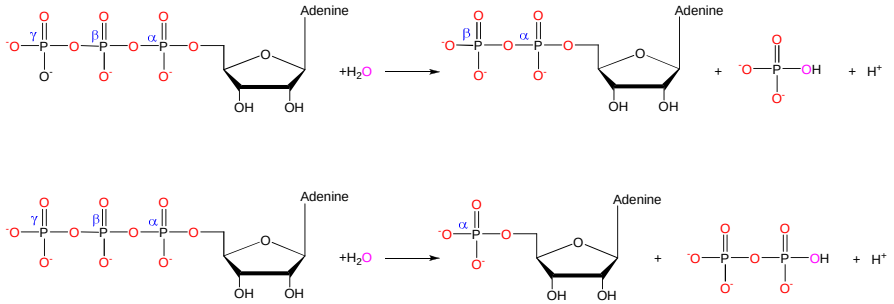
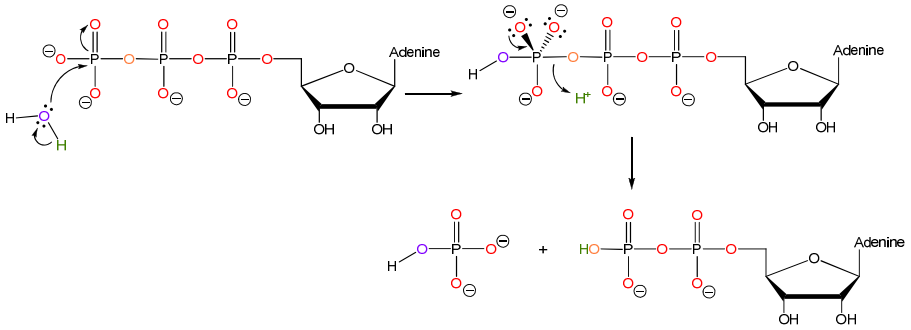
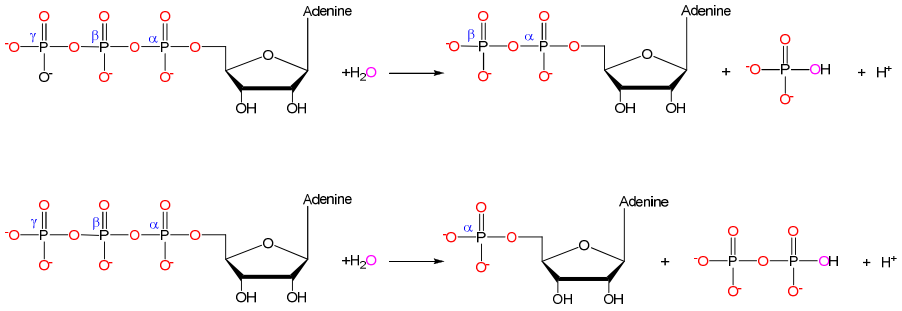


ATP hydrolysis (nonenzymatic)



ATP hydrolysis and P_i transfer



The simple hydrolysis of ATP does nothing but generate heat. To be beneficial, the free energy of ATP hydrolysis should be coupled to an endergonic reaction to make that reaction thermodynamically favorable

Ways in which the free energy of ATP hydrolysis is productively used in cellular processes

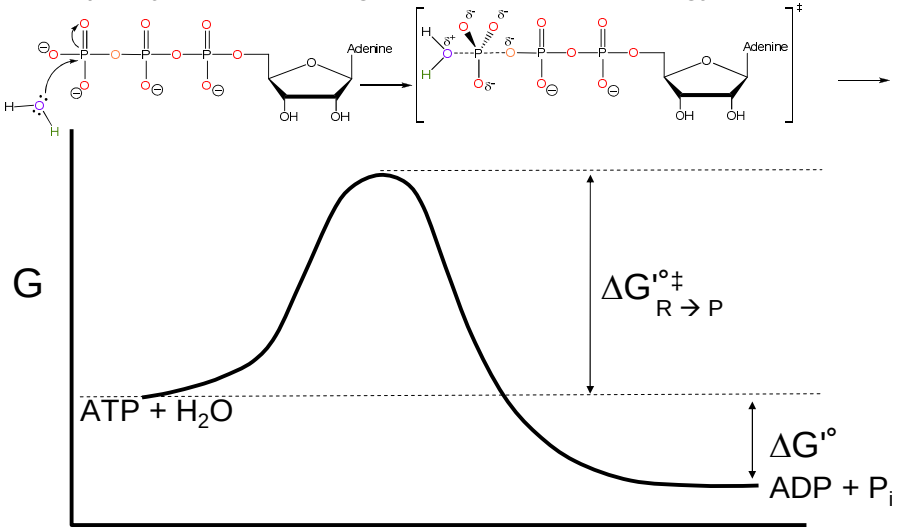
- generation of a phosphorylated organic compound used in metabolism
  - e.g., glucose catabolism involves initial phosphorylation reactions
  - e.g., convert poor leaving group to good leaving group for biosynthesis
- phosphorylation of amino acid side chains for various purposes
  - regulation of enzyme activity
  - subsequent hydrolysis to induce conformational change and perform mechanical work
- inducing conformational changes via direct ATP hydrolysis to perform mechanical work (muscles)
- etc

What is the free energy of hydrolysis of ATP under cellular conditions?

E. coli: 7.90 mM ATP, 1.04 mM ADP, 6.70 mM phosphate

Bioenergetic utility of phosphoryl transfer reactions

- Kinetic stability to nonenzymatic hydrolysis (neutral pH)
- Capacity to release large amounts of free energy



High energy compounds

- Contain one or more "high energy bond"
- High energy bond: a bond whose hydrolysis releases a large amount of free energy (>25 kJ/mol) ~

Standard free energies for hydrolysis of selected phosphoryl groups

Reaction (not necessarily charge-balanced)	$\Delta G'^{\circ}$ (kJ/mol)
phosphoenolpyruvate + H_2O → pyruvate + P_i	-61.9
1,3-diphosphoglycerate + H_2O → 3-phosphoglycerate + P_i	-49.3
ATP + H_2O → AMP + PP_i	-45.6
Phosphocreatine + H_2O → creatine + P_i	-43.0
ADP + H_2O → AMP + P_i	-32.8
ATP + H_2O → ADP + P_i	-30.5
Glucose-1-phosphate + H_2O → glucose + P_i	-20.9
PP_i + H_2O → 2 P_i	-19.2
Glucose-6-phosphate + H_2O → glucose + P_i	-13.8
Glycerol-3-phosphate + H_2O → glycerol + P_i	-9.2

What makes the phosphoanydride bond of ATP a "high energy bond"?

- Product ionization: shift equilibrium to right
- Release of electrostatic repulsion
- Resonance stabilization of hydrolysis products
- Solvation energy, phosphoanhydride vs. inorganic phosphate

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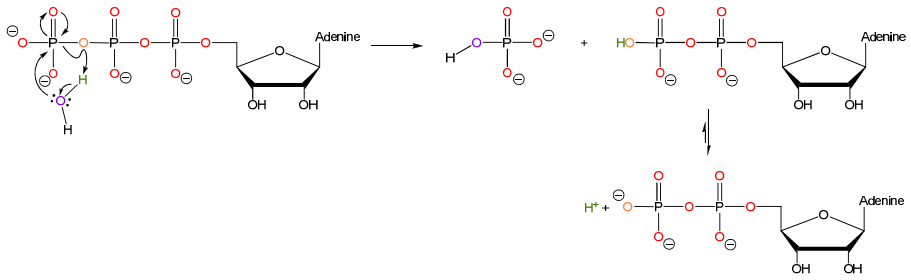

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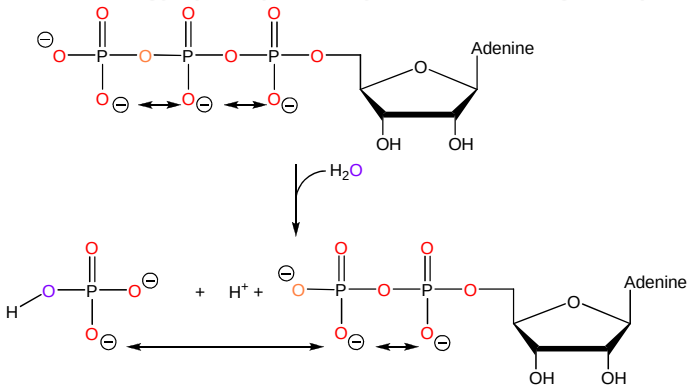

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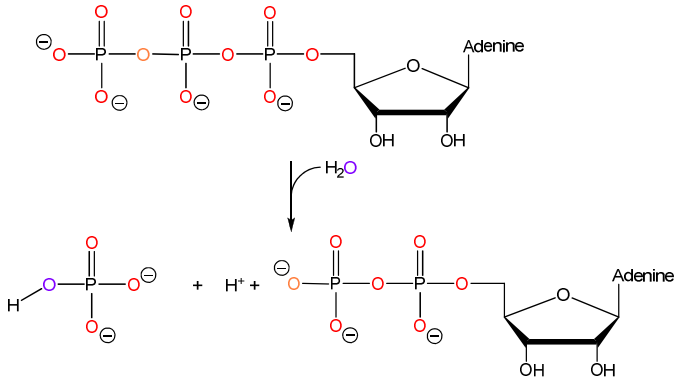
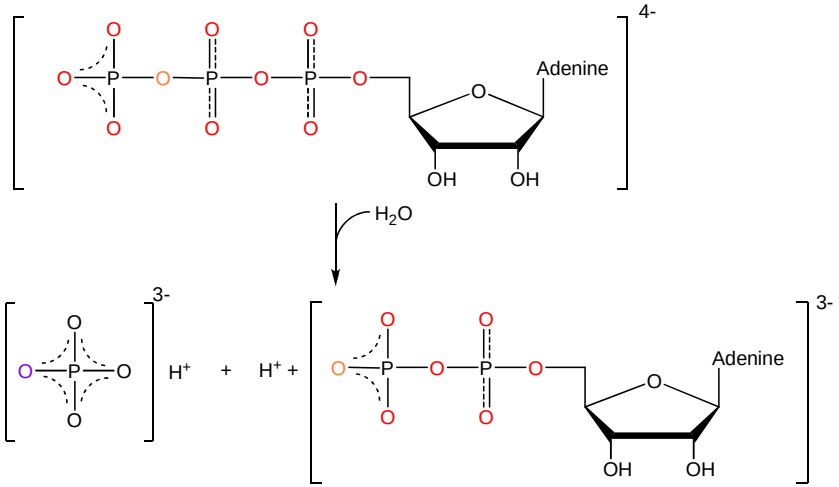


What makes the phosphoanydride bond of ATP a "high energy bond"?

- Resonance stabilization of hydrolysis products
- The more resonance structures that can be drawn, the more stable the compound

Number of resonance contributors in ATP and hydrolysis products



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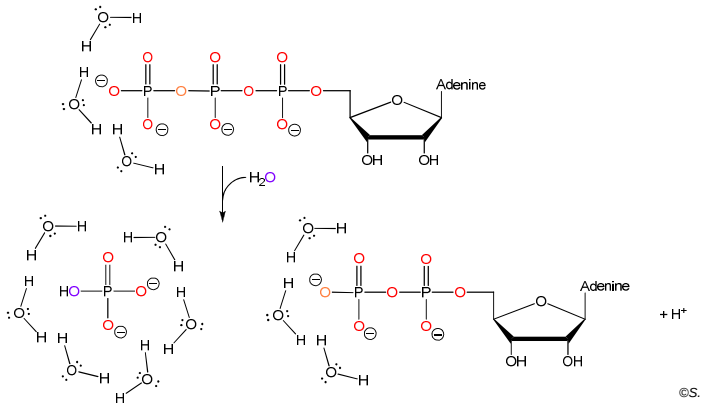
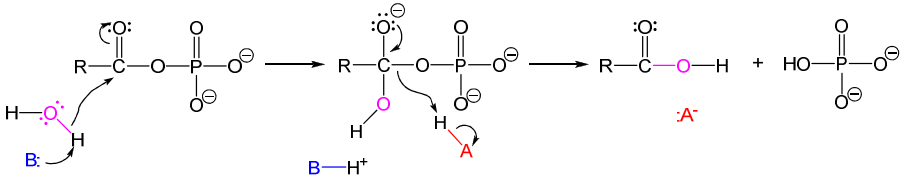


Other “high energy” compounds

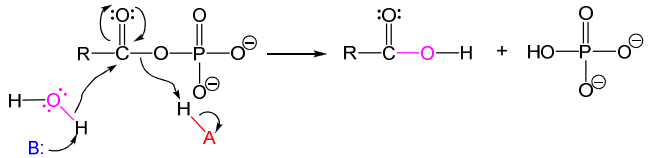
- Acyl phosphates
- Enol phosphates
- Phosphoguanidines
- thioesters

Acyl phosphates

- Note how hydrolysis of an acyl phosphate involves attack of hydroxide ion on the carbonyl C, not the P:



- Mechanism “shortcutting” the tetrahedral intermediate:



Contributors to the large negative free energy of acyl phosphate hydrolysis

- Product ionization: shift equilibrium to right
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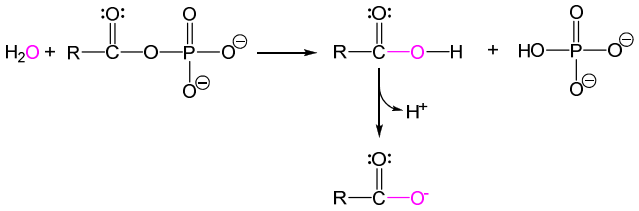

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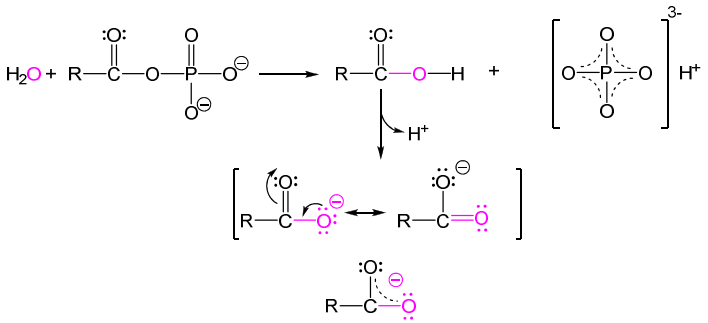

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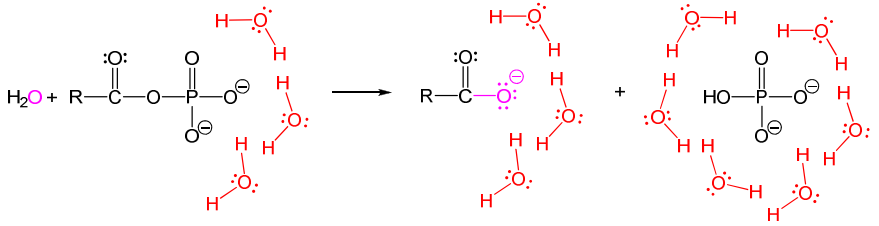
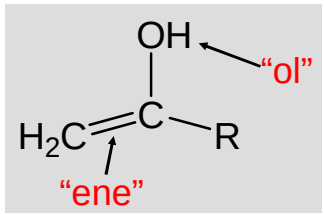
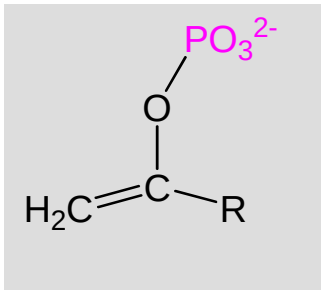


Enol phosphates



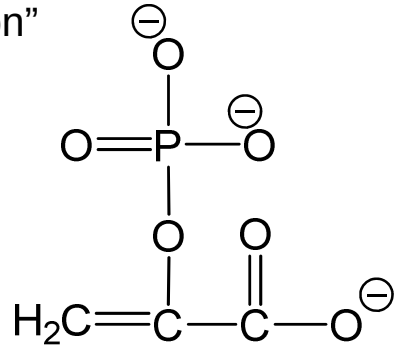
enol



enol phosphate

Enol phosphates

- An additional contributor to the driving force for enol phosphate hydrolysis is "keto-enol tautomerization"

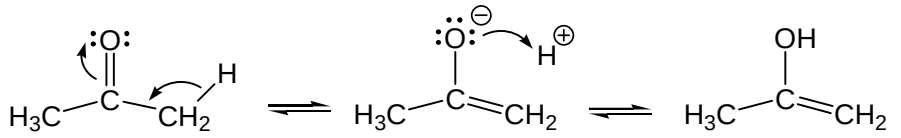


Tautomerization

- An intramolecular isomerization involving proton movement, resulting in interconversion of a single bond and a double bond
- Not the same as resonance stabilization
- Some tautomeric pairs:
  - ketone-enol
  - lactam-lactim
  - amide-imidic acid
  - enamine-imine

Keto-enol tautomerization

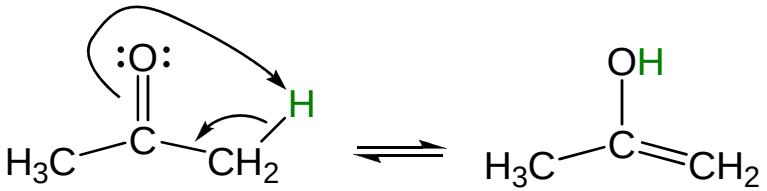
- Observed for ketones and aldehydes
- An equilibrium is established between the keto- and enol- isomers
- Equilibrium usually lies towards the ketone form
- Interconversion is concerted or through an "enolate" intermediate
- Keto-enol tautomerization in acetone:



keto tautomer enolate enol tautomer

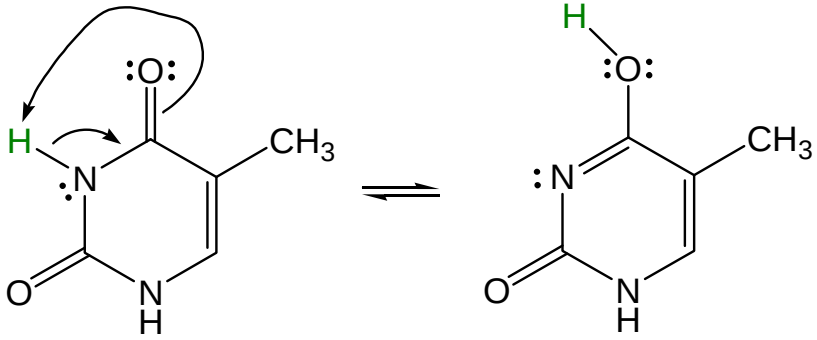
Keto-enol tautomerization

- Keto-enol tautomerization in acetone shown as a concerted mechanism:



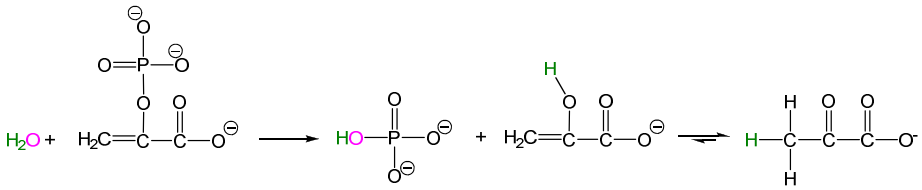
keto tautomer enol tautomer

Lactam-lactim tautomerization in nucleotide bases



lactam ring lactim ring

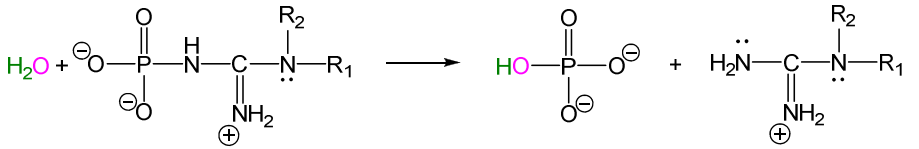
Enol phosphate hydrolysis



$\Delta G'^{\circ}$=-61.9 kJ/mol

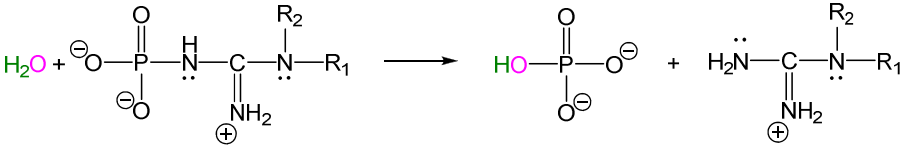
- **Product removal via enol to keto tautomerization**
- Release of electrostatic repulsion
- Resonance stabilization (phosphate)
- Solvation energy

Phosphoguanidines



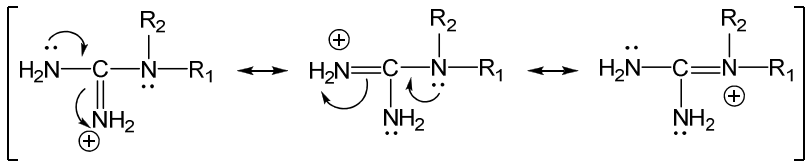
$\Delta G'^{\circ}_{PC}$=-43 kJ/mol

Phosphoguanidines



$\Delta G'^{\circ}_{PC}$=-43 kJ/mol

- Resonance stabilization of hydrolysis products
- Solvation energy



A nonphosphate high energy compound: acyl thioester

Summary of features that make a compound a “high energy” compound

- Electrostatic repulsion → charge separation
- Product ionization
- Product removed/stabilized by tautomerization
- Product stabilized by resonance
- Product solvation