# Enzymes in Organic Chemistry

Wed. March 18, 2009

# Enzymes

- Enzymes are biological catalysts. They increase the rate at which equilibrium is reached, but they do not affect the equilibrium.
- Enzymes differ from ordinary chemical catalysts:
  - ⇒ Enzyme catalyzed reaction rates are typically $10^6$ to $10^{12}$ greater than those of the uncatalyzed reactions.
  - ⇒ Enzyme catalyzed reactions occur under relatively mild conditions (physiological conditions).
  - ⇒ Enzymes often have tremendous specificity with regards to both their substrates and products.
  - ⇒ The activity of enzymes can be regulated by compounds other than their substrates and products.

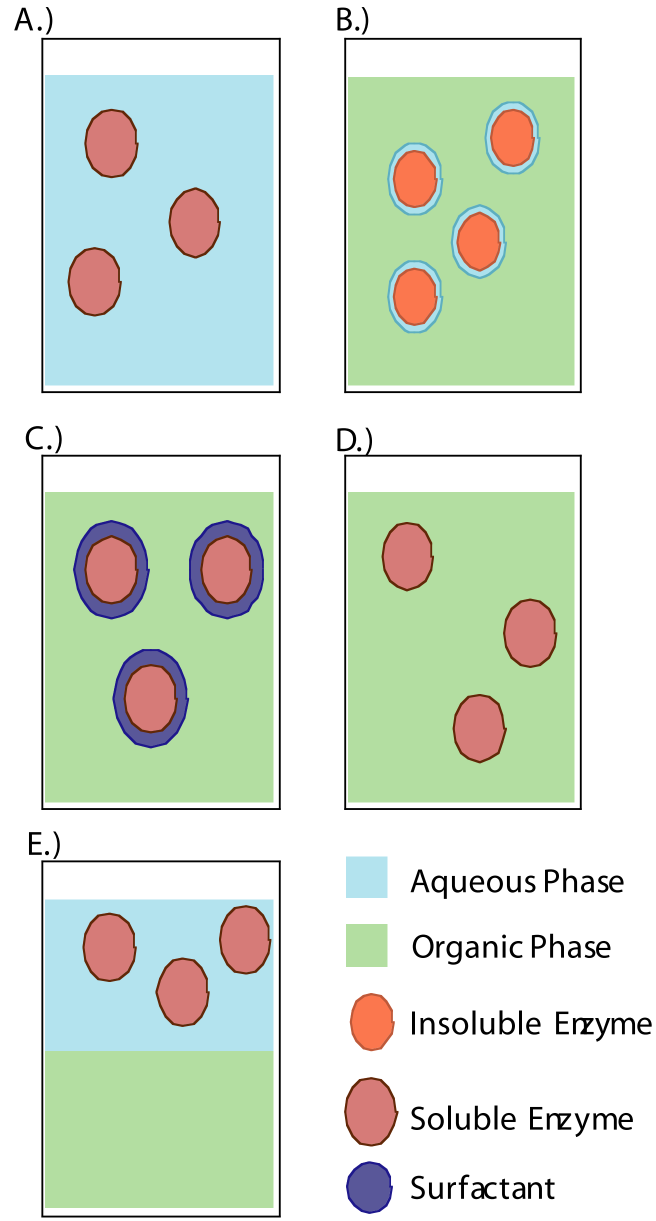
# Classes of Enzymes

| Classification | Type of Reaction Catalyzed |
|---|---|
| 1. Oxidoreductases | Oxidation–reduction reactions |
| 2. Transferases | Transfer of functional groups |
| 3. Hydrolases | Hydrolysis reactions |
| 4. Lyases | Group elimination to form double bonds |
| 5. Isomerases | Isomerization |
| 6. Ligases | Bond formation coupled with ATP hydrolysis |

Hydrolases and oxidoreductases are of particular interest.

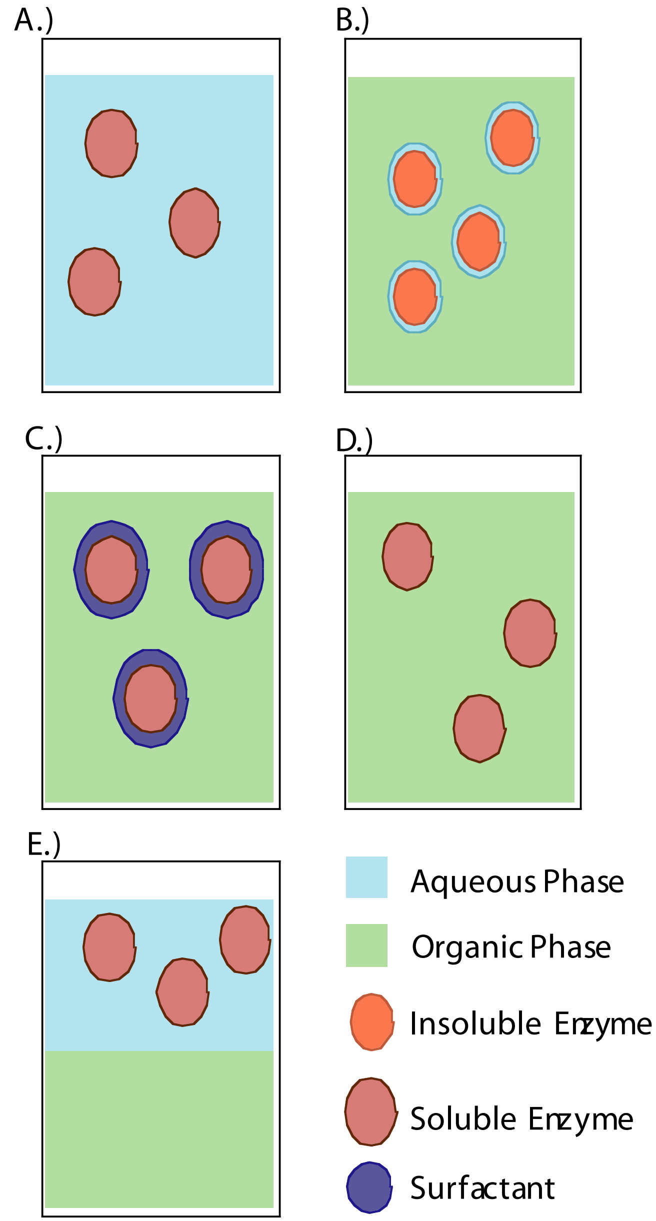
# Enzymes in Organic Solvents

- Enzymes have evolved to function in an aqueous environment.
- This has hampered their use in organic synthesis.
- Over the last 15 years it has been found that enzymes can also function in nonaqueous environments.
- Enzyme properties are affected by nonaqueous conditions and may differ significantly from those normally associated with the enzyme in an aqueous environment.



# Enzymes in Organic Solvents

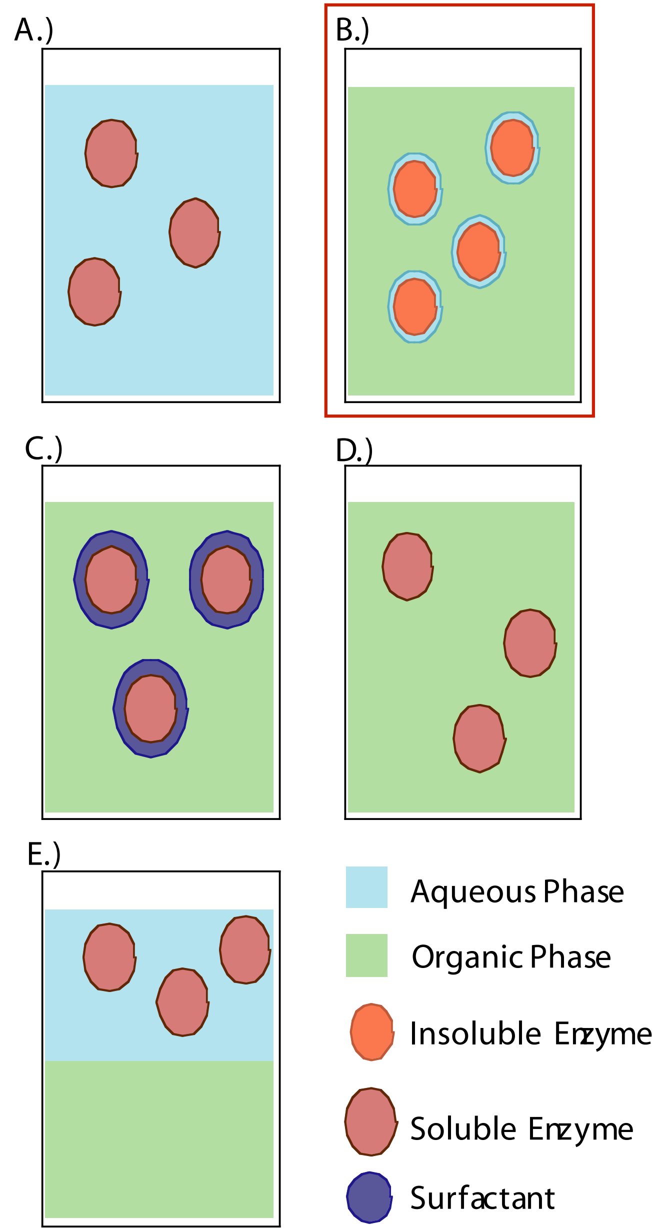
- Many enzymes demonstrate increased thermal stability in organic media.
- Lipase in nonaqueous conditions has been determined to be stable at 100°C for several hours. This is not true for the enzyme in aqueous solution
- It also demonstrated higher activity at 20°C than lipase in aqueous solution.
- This enhanced stability decreases as the water content increases.



# Enzymes in Organic Solvents

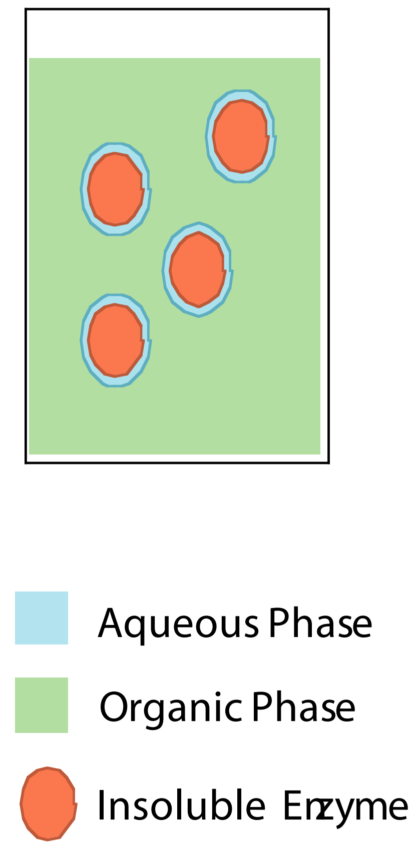
Various approaches for using enzymes in organic solvents:

A. Enzyme solvated in the normal aqueous environment.
B. Enzymes in a low-water solvent system. Enzyme is suspended in an organic solvent.
C. A microemulsion where the enzyme is encapsulated within a reverse micelle.
D. Monophasic cosolvent system where the enzyme is dissolved in a mixture of water and water miscible organic solvents.
E. A biphasic organic-aqueous system where the enzyme resides in the aqueous layer.



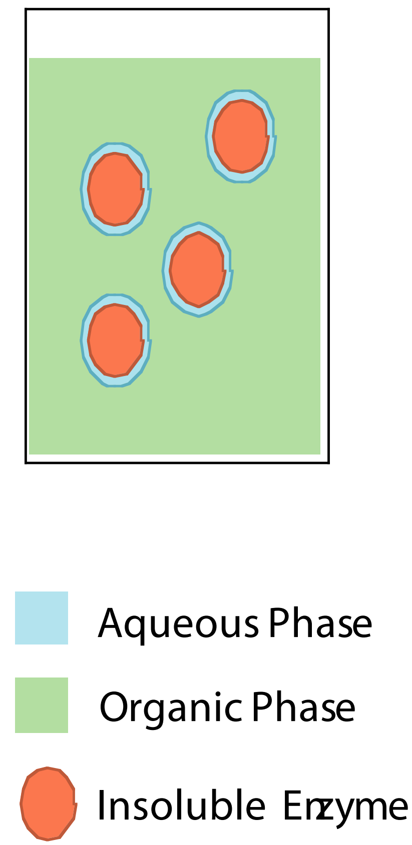
# Enzymes in a Low-Water Solvent System

- In organic media, enzymes require water for catalytic activity.
- In general, enzymatic catalysis improves with increased hydration (refering to water associated with protein, not water content of system as a whole).
- The degree of hydration required depends on the enzyme and the organic solvent.
  - ⇒ Lipases appear to require very few water molecules in order to be active.
  - ⇒ Some enzymes (such as chymotrypsin) are active when associated with 50 or fewer water molecules.
  - ⇒ Others (such as alcohol dehydrogenase) require sufficient water molecules for a monolayer to form.



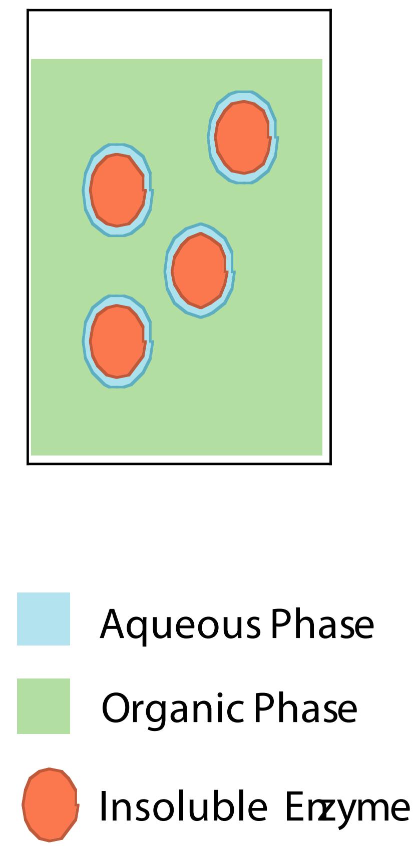
# Enzymes in a Low-Water Solvent System

- Under anhydrous conditions, the charged groups on the protein surface may interact tightly with each other, resulting in an inactive "locked" conformation.
- The presence of water molecules may increase enzyme flexibility allowing the enzyme to be catalytically active.
- The nature of the organic solvent also influences the behavior of suspended enzymes.
  - ⇒ Enzyme activity
  - ⇒ Substrate specificity
  - ⇒ Regioselectivity
  - ⇒ Enantioselectivity
  - ⇒ Prochiral selectivity



# Enzymes in a Low-Water Solvent System

- Polar organic solvents are capable of stripping the hydrating water molecules from the protein surface, decreasing enzyme activity.
- Hydrophobic solvents are less likely to disrupt or distort the aqueous layer surrounding the enzyme, promoting increased enzyme activity.
- Solvent may also affect enzyme activity by direct interaction with the substrates or products.
- The solvent may affect the effective substrate or product concentrations (particularly important in enzymes prone to substrate or product inhibition.



# Enzymes in Organic Solvents

- Enzymes are generally insoluble in organic solvents (DMSO being an exception) allowing for easy recovery of enzyme after use.
- One approach to nonaqueous biocatalysis is to utilize the enzyme as a fine powder suspended in the organic solvent.
- Another approach is to immobilize the enzyme by adsorbing it onto an inert support such as celite or porous silica.
- **Enzyme preparation, can affect enzyme properties and activity.**

# Suspended Enzyme

- **Enzyme powders suspended in organic solvents tend to be more rigid than when in an aqueous solution.**
- It has been proposed that he unique properties associated with enzymes in a nonaqueous environment is due in large part to this increased rigidity.
- The enzymes used in organic solvents are generally either lyopholyzed or precipitated from an aqueous solution with a pH optimal for enzymatic activity.
- **In the solid powder form, the ionogenic groups of the enzymes appear to retain the ionization state associated with the pH of the parent aqueous solution**.
- The pH associated with optimal performance in the organic suspension may differ from that of the enzyme in aqueous conditions. This shows some dependence on the organic solvent.

Comparison of alcohol dehydrogenase activities

| pH | Aqueous Solution | Nonaqueous Solution (heptane) |
|---|---|---|
| 2.0 | | maximum activity |
| 7.5 | maximum activity | |
| 11.0 | | |

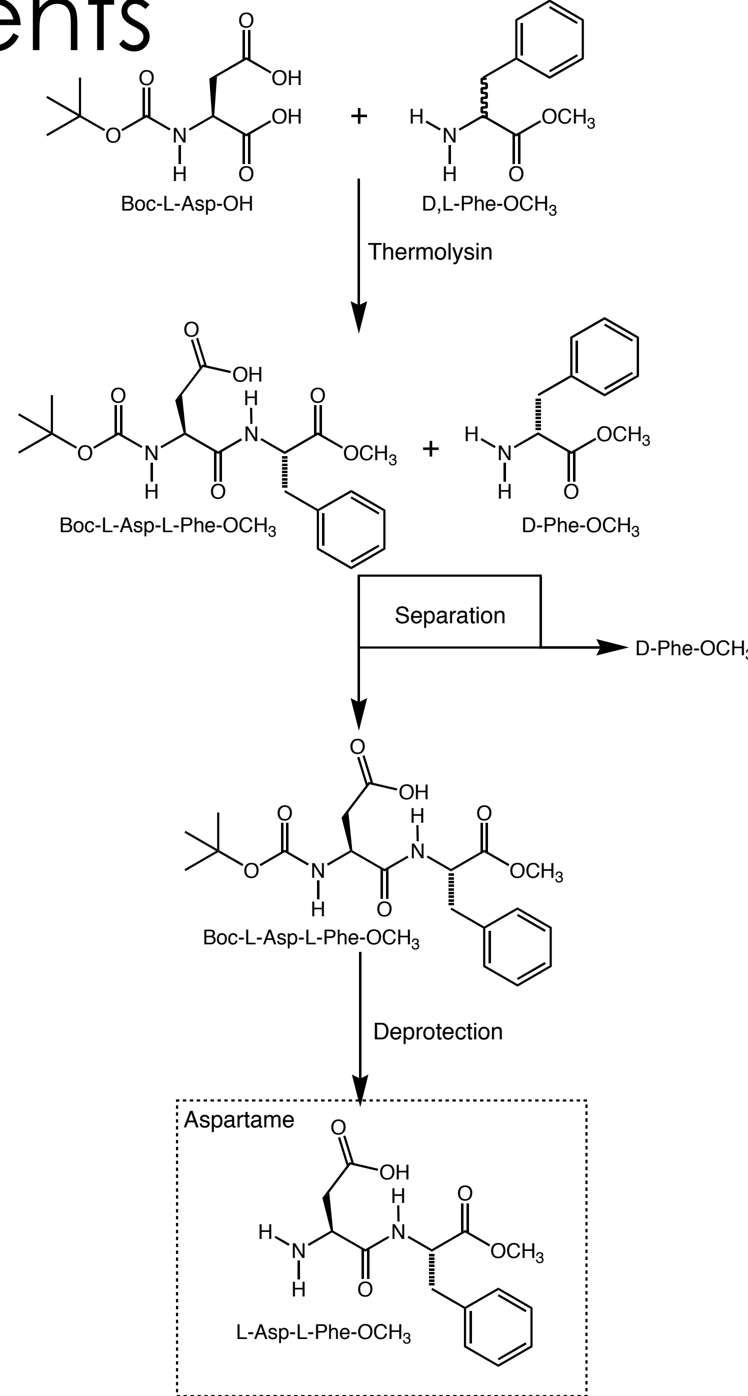
# Adsorbed Enzyme

- **Selection of support material is important**.
- The support (depending on its hydrophobicity) can influence the effective substrate/product concentration in the vicinity of the enzyme active site.
- The support can also affect enzyme hydration. Enzymes adsorbed on a hydrophobic support demonstrate increased activity due to improved hydration.
- The support can also affect the kinetics of an enzyme-catalyzed reaction.
- The porosity of the support can affect substrate/ product diffusion and degree of enzyme loading on the solid support.

# Enzymes in Organic Solvents

*Hydrolases*

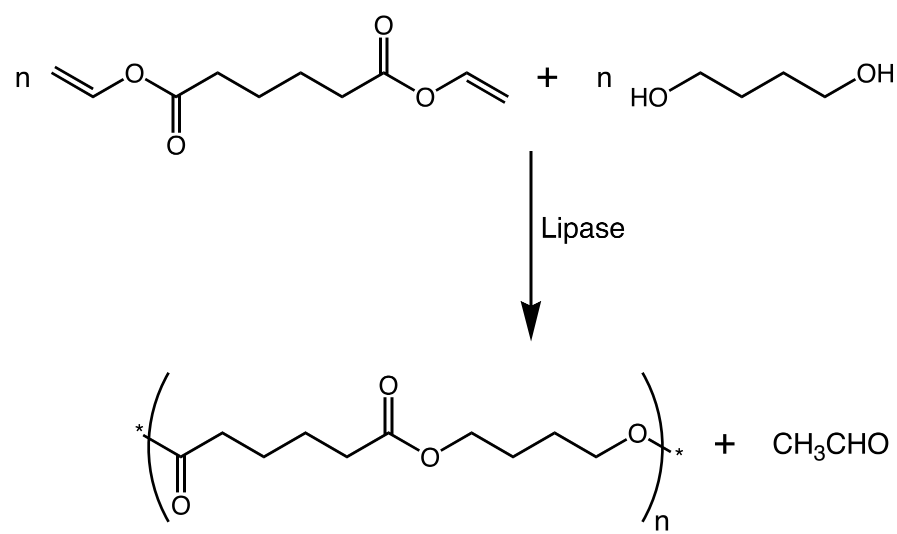
- Under nonaqueous conditions hydrolases (lipases and proteases) catalyze esterification, transesterification and amide condensation reactions rather than hydrolytic reactions.
- In a water-restricted environment the equilibrium of amide hydrolysis is shifted towards amide bond formation.
- Demonstrated in the enzymatic synthesis of the dipeptide L-Asp-L-Phe-$OCH_3$ (aspartame) using thermolysin.



# Enzymes in Organic Solvents

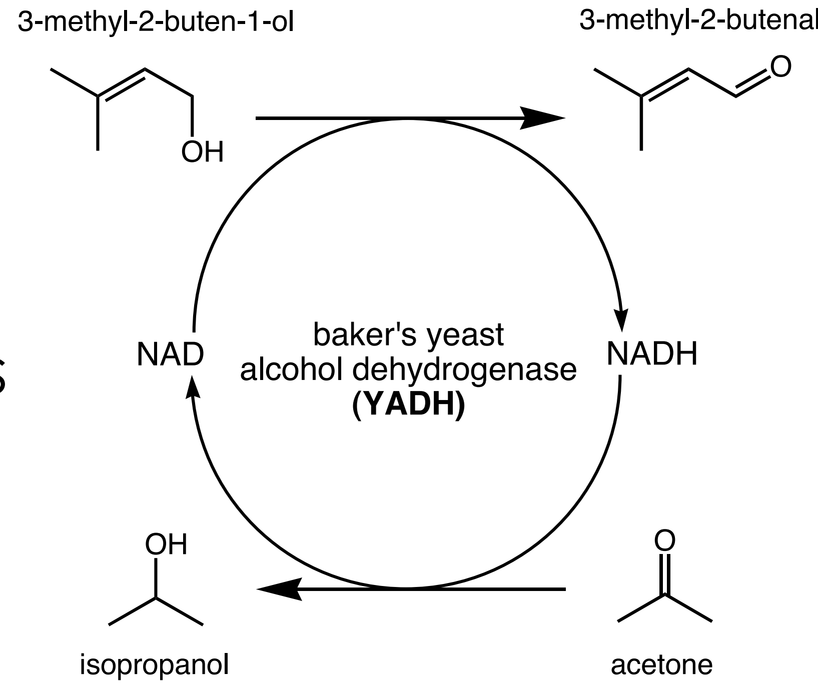
## *Hydrolases*

- Use of hydrolases in organic solvents allows the enzymatic synthesis of polymers such as polyesters and polyacrylates.
- This is not possible under aqueous conditions due to low solubility of monomers and/or the polymer product.
- Use of enzymes to fabricate polymers capitalizes on the selectivity associated with enzyme catalyzed reactions.
- Enzymes can be used in conjunction with traditional catalysts for chemo-enzymatic synthesis of polymers.



# Enzymes in Organic Solvents
## *Oxidoreductases*

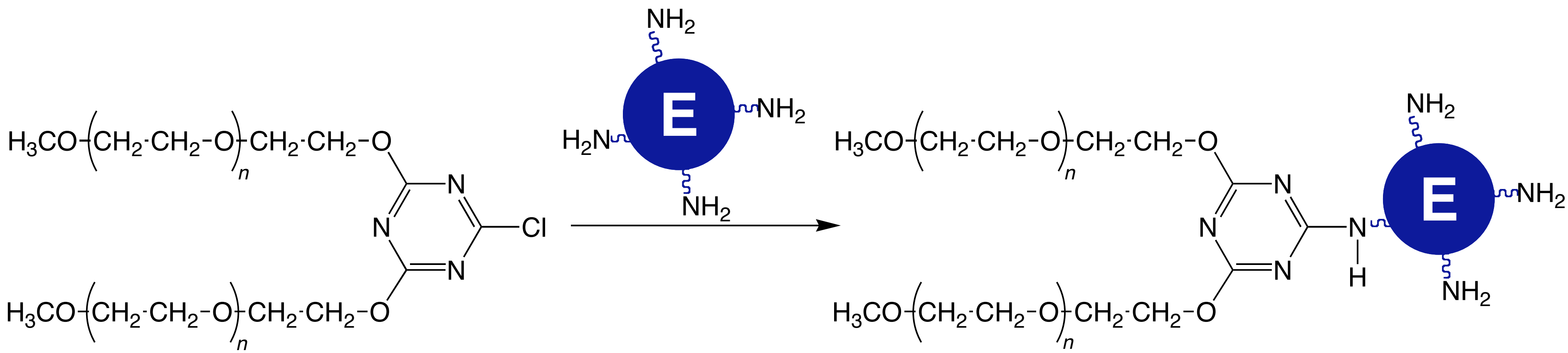
- As in the case of hydrolases, the catalytic activities of oxidoreductases are affected by a nonaqueous environment.
- Most oxidoreductases require cofactors in order to carry out redox reactions.
- In order to function properly, it is necessary to regenerate the cofactor.
- YADH in heptane efficiently oxidizes 3-methyl-2-bute-1-ol to the corresponding aldehyde.
- The cofactor (NAD+) can be recycled *in situ* by the addition of acetone.



# Enzymes in Organic Solvents

- Attachment of amphiphilic polymers such as PEG to enzymes can be used to generate enzyme constructs that are soluble in organic solvents. (PEG-modified enzymes are soluble in organic solvents in the mg/ml range.)
- The specific activities of such PEG-enzyme constructs are often similar to those of the free enzyme under aqueous conditions.
- The PEG-modified enzymes generally demonstrate greater stability than the parent enzymes.
- May also see changes in the optimal temperature for enzyme activity.
  - ⇒ PEG-lipase catalyzing ester exchange reaction under nonaqueous conditions---> optimal temp. 70°C
  - ⇒ Lipase catalyzing the hydrolysis of esters under aqueous conditions ---> optimal temp. 45°C.

# PEGylation of enzymes using a 2,4-bis(mPEG)-6-s-chlorotriazine




($mPEG_2$-chlorotriazine)