M2/L5: Enzyme immobilization

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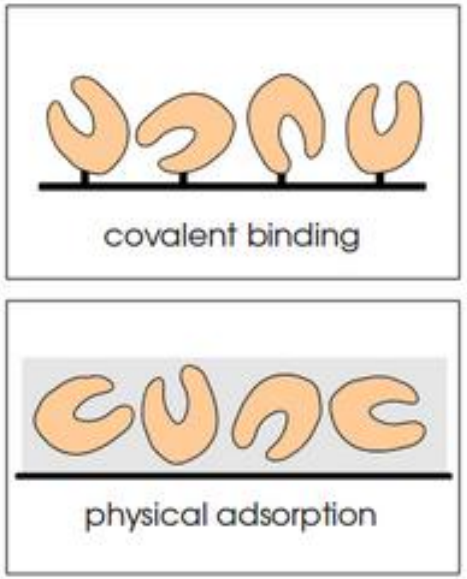
Enzyme Stability

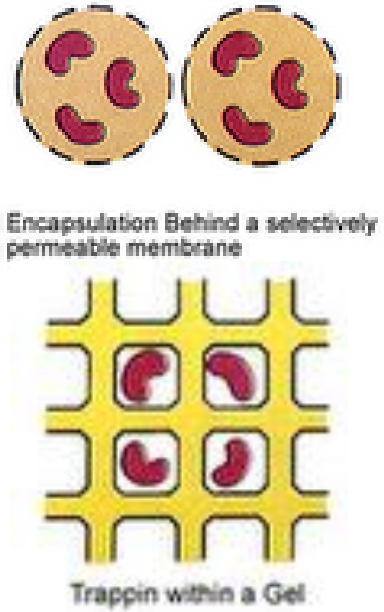
Denaturant Type	Target	End Product
Temperature	Hydrogen bonds	Highly disordered structure Aggregates
Acids	Buried uncharged group (histidine, peptide bonds)	Random coil
Alkali	Buried uncharged groups (tyrosine, cysteine)	Random coil
Salts	Polar and non-polar groups	Highly disordered structure
Solvents	Non-polar groups	Highly disordered peptide with large helical regions
Surfactants	Hydrophobic domains	Disordered with large helical regions

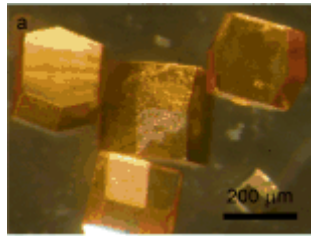
Enzyme Immobilization

- Immobilization is the most widely used technique. This is due to:
  - Ease of separation of the enzyme from the reaction products
  - Enzyme reuse is economically advantageous (reduction in waste and catalyst production)
  - Stabilization of the enzyme

Different types of immobilization

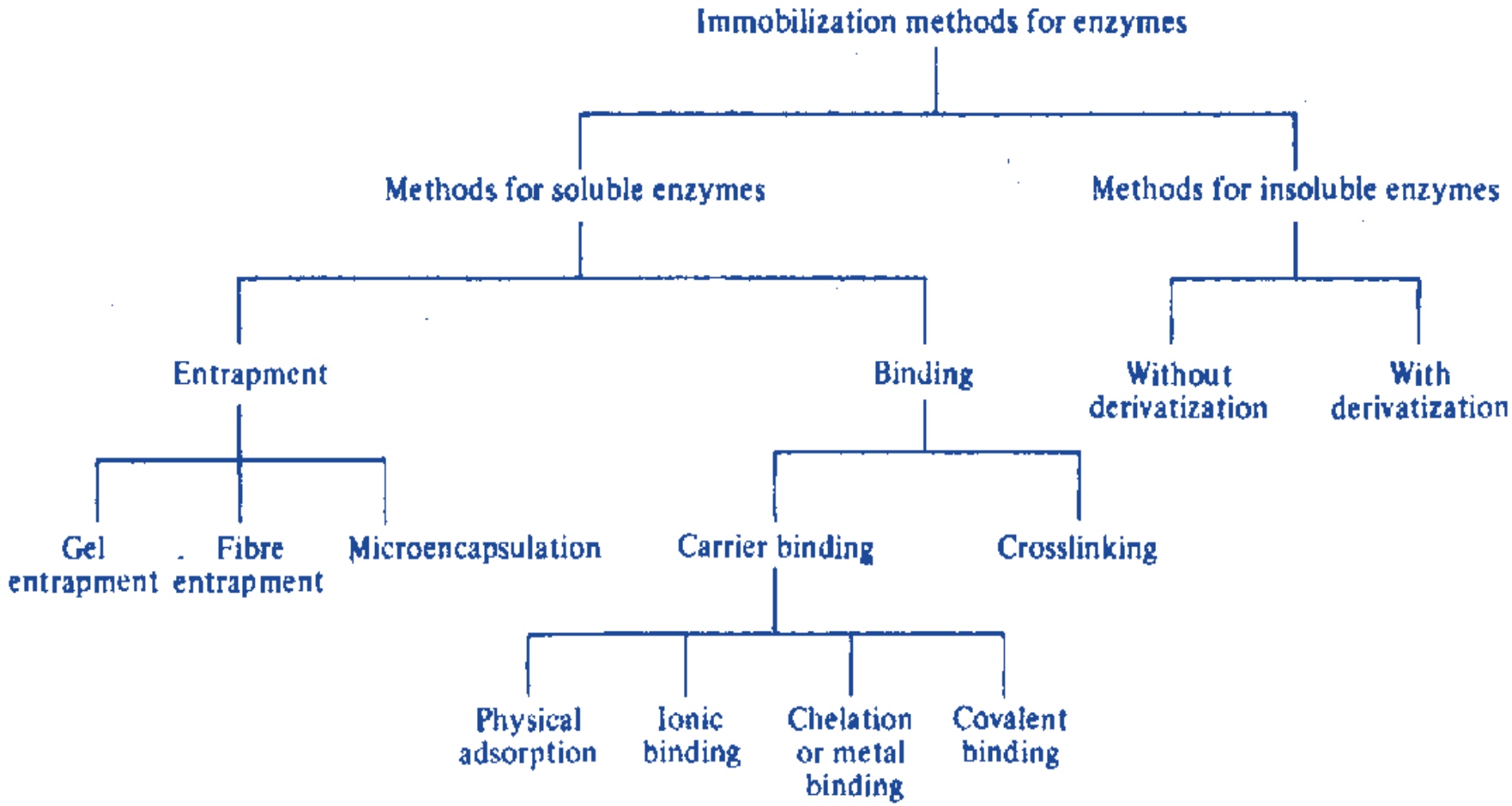






cross-linked enzyme aggregate (**CLEA**)

Types of immobilization method



Enzyme entrapment

- Gel entrapment places the enzyme within the interstitial spaces of crosslinked, water-insoluble polymer gels. E.g., Polyacrylamide gels & Polysaccharides.
- Variations of pore size result in enzyme leakage, even after washing. The effect of initiator used in polyacrylamide gels can be problematic.

Entrapment in microcapsule

Microencapsulation encloses enzymes within spherical, semi-permeable membranes of 1-100 μm diameter.

Urethane prepolymers, when mixed with an aqueous

enzyme solution crosslink via urea bonds to generate membranes of varying hydrophilicity.

Alternatively, photo-crosslinkable resins can be gelled by UV-irradiation.

Advantage of Entrapment

- Enzymes are immobilized without a chemical or structural modification. A very general technique.

Disadvantage of Entrapment

- High molecular weight substrates have limited diffusivity, and cannot be treated with entrapped enzymes.

Matrix Materials used in Entrapment

Organics: polysaccharides, proteins, carbon, vinyl and allyl polymers, and polyamides. e.g. Ca-alginate, agar,
K-carrageenin, collagen

Immobilization procedures:

Enzyme + polymer solution → polymerization
→ extrusion/shape the particles

Inorganics: activated carbon, porous ceramic.

Shapes: particle, membrane, fiber

Challenges in Entrapment Method

- enzyme leakage into solution
- diffusional limitation
- reduced enzyme activity and stability
- lack of control micro-environmental conditions.

It could be improved by modifying matrix or membrane.

Carrier Binding or Surface Immobilization

Attachment of an enzyme to an insoluble carrier creates an active surface catalyst. Modes of surface attachment classify carrier methods into physical adsorption, ionic binding and covalent binding.

Physical Adsorption: Enzymes can be bound to carriers by physical interaction such as hydrogen bonding and/or van der Waal's forces.

- the enzyme structure is unmodified
- carriers include chitosan, acrylamide polymers and silica-alumina
- binding strength is usually weak and affected by temperature and the concentration of reactants.

Ionic Binding: Stronger enzyme-carrier binding is obtained with solid supports containing ion-exchange residues.

- cellulose, glass-fibre paper, polystyrene sulfonate
- pH and ionic strength effects can be significant

Adsorption

Covalent immobilization

Covalent attachment of soluble enzymes to an insoluble support is the most common immobilization technique.

Amino acid residues not involved in the active site can be used fix the enzyme to a solid carrier

Advantages:

1. Minimal enzyme leaching from the support results in stable productivity
2. Surface placement permits enzyme contact with large substrates

Disadvantages:

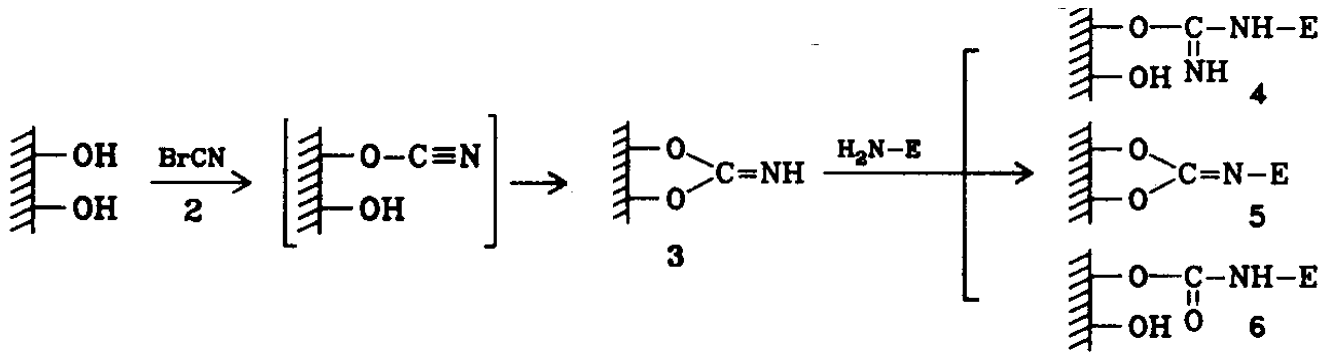
1. Partial modification of residues that constitute the active site decreases activity
2. Immobilization conditions can be difficult to optimize (often done in the presence of a competitive inhibitor)

- Covalent Binding: covalent bonds

Carriers: polymers contain amino, carboxyl, sulfhydryl, hydroxyl, or phenolic groups.

- Loss of enzyme activity
- Strong binding of enzymes

•Cyanogen bromide activates supports with vicinal hydroxyl groups (polysaccharides, glass beads) to yield reactive imidocarbonate derivatives:



•Other methods include diazo coupling, alkylation, etc.

Carrier-free immobilization

Extensive research over the past 20 years has been performed for the immobilization of enzymes on carriers.

However, carrier-free immobilization has several advantages:

- Elimination of solid support carrier and reagents
- Ease of preparation
- Reduced activity loss
- Increased space-time yield

Immobilization by Crosslinking

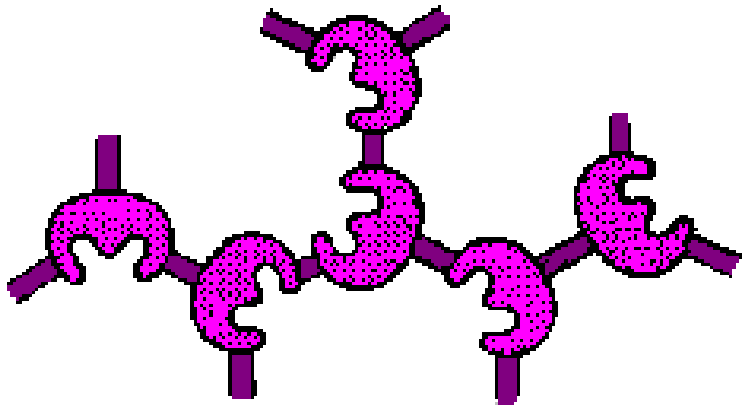
Bi- or multi-functional compounds serve as reagents for intermolecular crosslinking of enzymes,

creating insoluble aggregates that are effective heterogeneous catalysts.

Reagents commonly have two identical functional groups which react with specific amino acid residues.

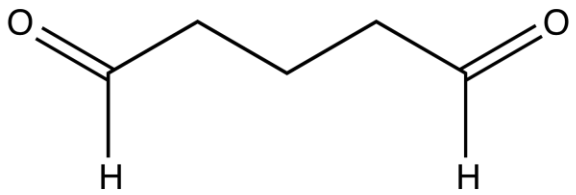
Common reagents include glutaraldehyde, carbodimide and diisocyanates,

Involvement of the active site in crosslinking can lead to great reductions in activity, and the gelatinous nature of the product can complicate processing.

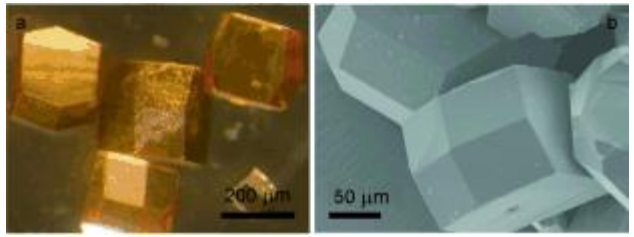


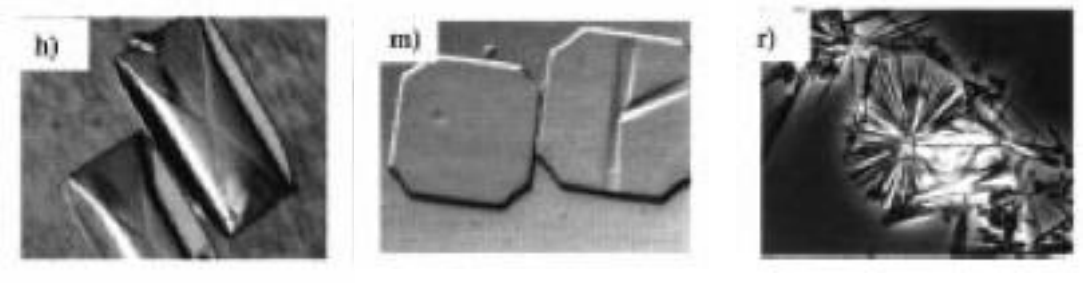
Cross-Linked Enzyme Crystals (CLECs)

In the 1960's cross-linking reagents were first used to stabilize enzyme crystals for crystallography.



Glutaraldehyde





Comparison between methods

Comparison of the attributes etc. of different classes immobilization techniques

Characteristic	Crosslinking	Physical adsorption	Ionic binding	Chelation or metal binding	Covalent binding	Entrapment
Preparation	Intermediate	Simple	Simple	Simple	Difficult	Difficult
Binding force	Strong	Weak	Intermediate	Intermediate	Strong	Intermediate
Enzyme activity	Low	Intermediate	High	High	High	Low
Regeneration of carrier	Impossible	Possible	Possible	Possible	Rare	Impossible
Cost of immobilization	Intermediate	Low	Low	Intermediate	High	Intermediate
Stability	High	Low	Intermediate	Intermediate	High	High
General applicability	No	Yes	Yes	Yes	No	Yes
Protection of enzyme from microbial attack	Possible	No	No	No	No	Yes

Immobilized enzymes!

- ***Advantages***
  - Retention in reactor
  - Separation from reaction components is facilitated
  - Usable in a wide range of reactor configurations
  - High catalytic loadings
  - Enhanced stability toward T, pH, solvent, etc.
  - Modified selectivities
- ***Disadvantages***
  - Mass-transfer limitations
  - Loss of activity upon immobilization
  - Impractical for solid substrates

Thank you