CHAPTER 3
IMMOBILIZATION OF BIOLOGICAL SENSING ELEMENTS

Learning Objectives:

At the end of this chapter you should be able to:

1. State five types of immobilization methods used to make a viable biosenser

2. Illustrate the methods that can be used to immobilize biomolecules to a transducer for the construction of a viable biosensor.

IMMOBILIZATION

✓ The basis of a biosensor is the close contact between microorganisms and the transducer.

✓This can be achieved through a process known as immobilization.

✓ The technology plays an important role and the choice of immobilization technique is critical as it influences the response, and operational stability of biosensors.

IMMOBILIZATION

Importance of Immobilization

✓ To make a viable biosensor, the biological component has to be properly attached to the transducer ⇒ *Immobilization*

Definition of immobilization (in biotechnology)

"The technique used for the physical or chemical fixation of cells*, organelles, enzymes, or other proteins (e.g. monoclonal antibodies) onto a solid* support, into a solid matrix or retained by a membrane, in order to increase their stability and make possible their repeated or continued use". (IUPAC)

IMMOBILIZATION

5 main methods

1. **Adsorption**
2. **Entrapment**
3. **Microencapsulation**
4. **Cross-linking**
5. **Covalent bonding**

ADSORPTION

- ✓ Adsorption – physical method of immobilization
- ✓ Many substances adsorb enzymes on their surfaces (alumina, charcoal, clay, cellulose, kaolin, silica gel, glass & collagen, carbon pellets & advanced material such as carbon nanotubes (CNTs)
- ✓ Eg. microbial cells are immobilized by simple absorption by placing the cells on a porous cellulose membrane
- ✓ Eg. Enzymes or tissue are mixed with graphite powder and liquid parrafin to produce an enzyme or tissue carbon paste electrode (bananatrode for dopamine detection)

ADSORPTION

ADVANTAGES	DISADVANTAGES
No reagents required / minimal preparation	Attachment is *weak* thus leaching of enzyme from support (H-bonding, van der Waals forces)
No clean-up step	Adsorbed biomaterial very susceptible to *environmental changes* (pH, temperature, ionic strength)
Less disruption to the enzymes	Suitable for *short-term* investigations lifetime limited

MICROENCAPSULATION

- ✓ Encapsulation – physical method of immobilization
- ✓ Method used with the first glucose biosensor on the oxygen electrode. (*First Generation*)
- ✓ Biomaterial held in place behind an inert membrane – close contact between biomaterial and transducer.

MICROENCAPSULATION

Main types of membranes used :

- *cellulose acetate* (dialysis membrane) – excludes proteins and slows transportation of interfering species such as ascorbate
- *polycarbonate* (*Nucleopore*) – synthetic non-permselective material
- *collagen* – a natural protein
- *PTFE* : polytetrafluoroethylene (trade name Teflon) – a synthetic polymer selectively permeable to gases such as oxygen
- *Nafion*
- *Polyurethane*

MICROENCAPSULATION

ADVANTAGES	DISADVANTAGES
Close attachment between biomaterial and transducer	Attachment is *weak* thus leaching of enzyme from support (H-bonding, van der Waals forces)
It is very adaptable	Encapsulated biomaterial very susceptible to *environmental changes* (pH, temperature, ionic strength)
It is very reliable (does not interfere with enzyme for eg)	Suitable for *short-term* investigations lifetime limited
Option of binding biological component to sensor via molecules that conduct electrons, eg., polypyrrole	

ENTRAPMENT

- ✓ Entrapment – physical method of immobilization
- ✓ Entrapment means physical enclosure of biomolecule in a small space
- ✓ A polymeric gel is prepared in a solution containing the biomaterial. The biomaterial is thus entrapped within the gel matrix
- ✓ Matrices commonly used are chemical polymers such as calcium alginate, carrageenan, polyacrylamide, and sol-gel

ENTRAPMENT

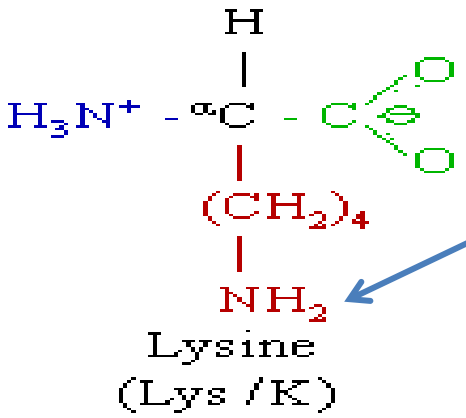
ADVANTAGES	DISADVANTAGES
Simplest method for biomolecule immobilization	Large barriers created - inhibiting diffusion of substrate this slows the reaction hence : Decreases response time of biosensor Lowers sensitivity Lowers detection limit
Gentle method that requires no chemical modification and only mild reaction condition is applied thus minimizing loss of bio-activity	Leakage of enzymes through pores- loss of bioactivity through the pores in the gel.

CROSS-LINKING

- ✓ Method uses *bifunctional agents* to bind the biomaterial to solid supports

What is a bifunctional agent?

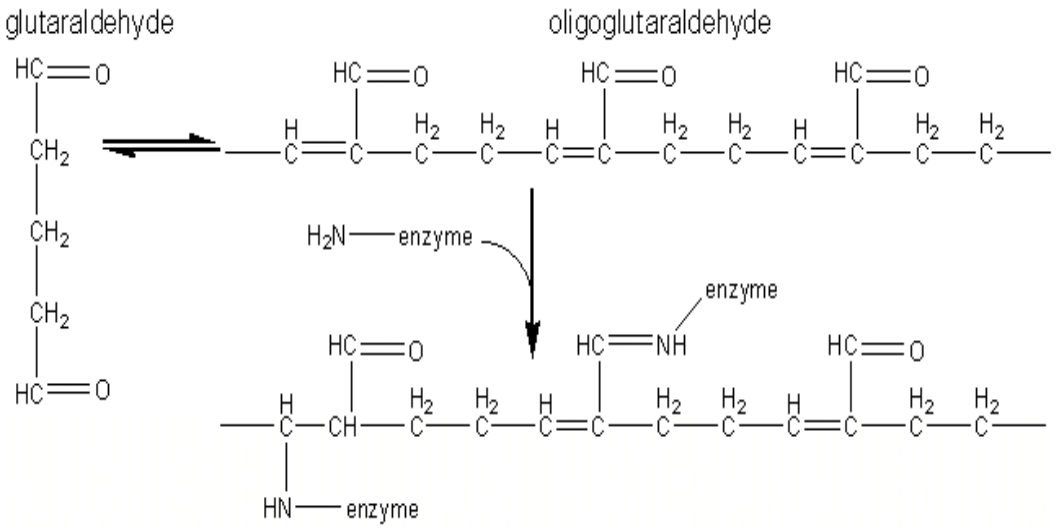
- ✓ A bifunctional compound links covalently to the *amine groups* of *lysine* or hydroxylysine in the protein molecules → chemical modification of enzymes/proteins.



amine group which will link to gluteradehyde

CROSS-LINKING

Immobilisation achieved by *intermolecular cross-linking* of the protein, either to other protein molecules or to functional groups on an insoluble support matrix.



CROSS-LINKING

ADVANTAGES	DISADVANTAGES
Stabilize the adsorption of biomolecules	*Expensive & insufficient* : can result in relatively *low enzymatic activity*
Simple and fast method	It causes damage to the enzyme (change conformation of active center of enzyme : significant loss of activity)
Cross-linking best *used in conjunction* with one of the other methods such as *adsorption* and *entrapment*.	It limits diffusion of the substrate
	There is poor rigidity (mechanical strength)

COVALENT BONDING

✓ Functional groups not essential for catalytic activity of enzyme can be covalently bonded to the support matrix (transducer or membrane).

✓ This method uses *nucleophilic* groups for coupling such as NH_2, COOH, OH, C_6H_5OH, SH (thiol group) and imidazole.

What are nucleophilic groups?

Groups which act by donating or sharing electrons.

COVALENT BONDING

Why are lysine residues the most useful groups for covalent bonding of enzymes to insoluble supports?

a. widespread surface exposure and high reactivity, especially in slightly alkaline solutions.

b. their rare involvement in the active sites of enzymes.

COVALENT BONDING

How can one be ensured that the catalytic activity of the enzyme is retained during immobilisation?

→ reducing the amount of enzyme bound in non-catalytic conformations but how can this be achieved?

→ Immobilisation of the enzyme in the presence of saturating concentrations of substrate, product or a competitive inhibitor ensures that the active site remains unreacted during the covalent coupling and reduces the occurrence of binding in unproductive conformations.

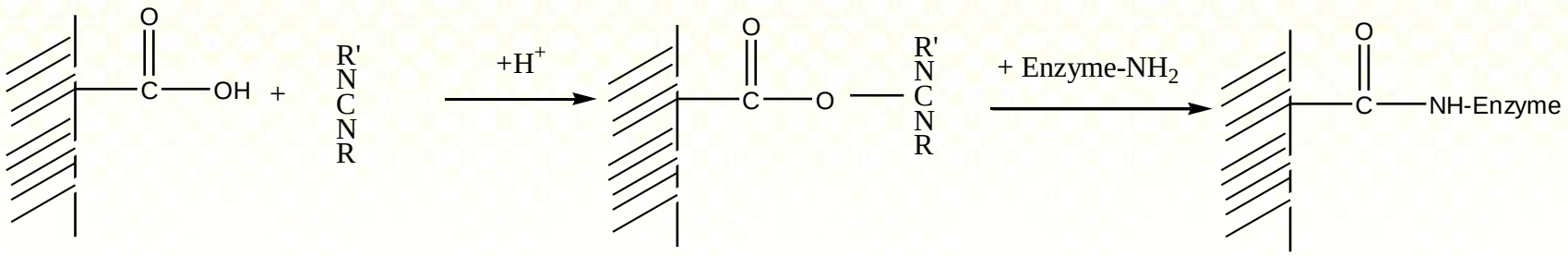
COVALENT BONDING

How can the activity of the immobilised enzyme be restored?

→ The activity of the immobilised enzyme can simply be restored by washing the immobilised enzyme to remove these molecules (i.e substrate). The active site of the immobilised enzyme is now again free to react with its substrate.

COVALENT BONDING

ADVANTAGES	DISADVANTAGES
Enzyme or biomaterial will not be released during use (minimal leaching)	Reactions need to be performed under mild conditions – low Temp, low ionic strength and pH in the physiological range.
Increased stability of biomaterial	More expensive and complex immobilization method
	Support needs to activated prior to immobilization



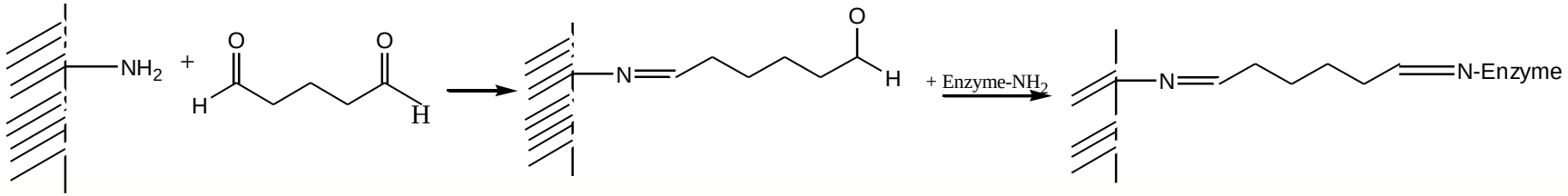
Carboxylic acid-containing support

Carbodiimide

Activated support

Immobilised enzyme

Activation of a carboxylic acid-containing support with a carbodiimide followed by enzyme coupling.



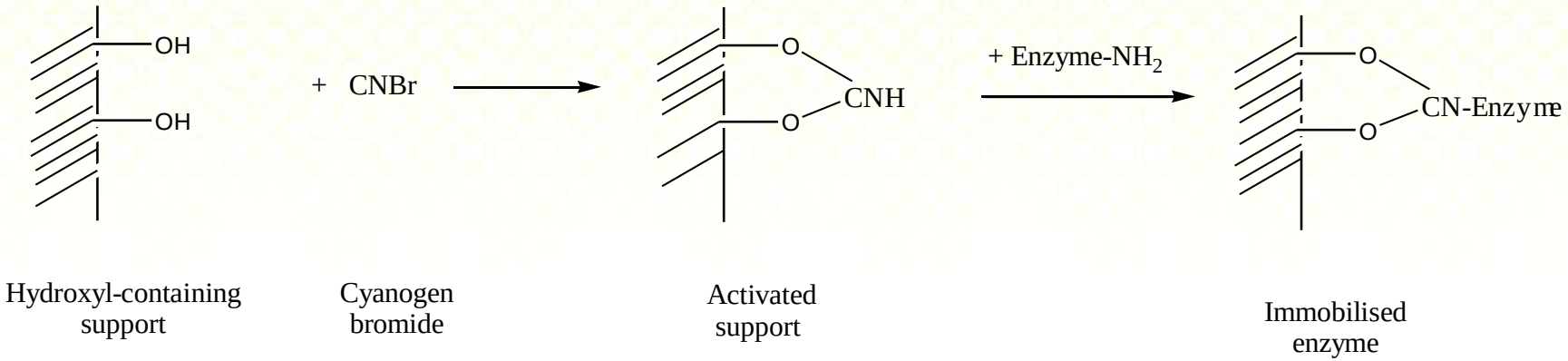
Amine-containing support

Gluteraldehyde

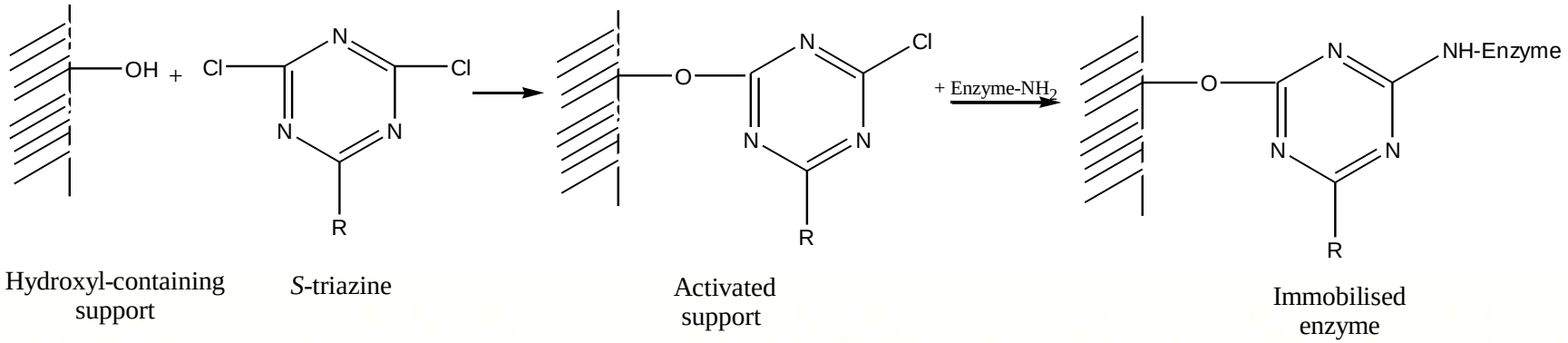
Activated support

Immobilised enzyme

Activation of an amine-bearing support with gluteraldehyde followed by enzyme coupling.



Hydroxyl-containing support | Cyanogen bromide | Activated support | Immobilised enzyme



Hydroxyl-containing support | *S*-triazine | Activated support | Immobilised enzyme

Enzyme immobilisation onto hydroxyl-containing supports via activation with cyanogen bromide (top) or *S*-triazine derivatives (bottom).