CHAPTER 2
BIOLOGICAL SENSING ELEMENTS

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Learning Objectives:

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

1. State six types of material that can used as biosensing elements

2. Discuss the advantages and disadvantages of each type of as biosensing element in relation to their use in biosensors.

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BIOSENSING ELEMENTS

- Bioreceptors/Bioelements/Biological Recognition Agents
- Key to specificity for biosensor technologies
- Responsible for binding analyte of interest to biosensor for measurement

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BIOSENSING ELEMENT	CATALYTIC	AFFINITY
Enzymes	√	
Tissue material	√	
Whole cells	√	
Antibody/Antigen		√
Nucleic acid (DNA)		√

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ENZYMES

✓proteins that *catalyze* (*i.e. accelerate*) *chemical reactions*. In these reactions, the *molecules* at the beginning of the process are called *substrates (specific to an enzyme)*, and the enzyme converts these into different molecules, the *products*.

✓most commonly used bioreceptor (glucose oxidase (Gox), urease, polyphenol oxidase (PPO))

✓ may be used in purified form

✓present in microbes / in slices of intact tissue

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ENZYMES

ADVANTAGES	DISADVANTAGES
Highly selective	Expensive (cost of source, extracting, isolating, purifying very high)
Have catalytic activity, thus improving sensitivity	Activity may be lost when immobilised on a transducer
Fairly fast acting	Tend to lose activity, due to deactivation after a relatively short period of time (unless stored under appropriate conditions)

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TISSUE MATERIAL

ADVANTAGES	DISADVANTAGES
Sensors have longer lifetime : enzymes more stable as they exist in their natural environment so less subject to degradation cheaper than purified enzymes	Tissues contain a multiplicity of enzymes so may not be as selective as purified enzymes – decreased substrate specificity
Cheaper than purified enzymes	Response time slower : more tissue material for substrate to diffuse through; this may also dilute effect of enzymes
May work when purified enzymes fail	

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WHOLE CELLS/MICROBES

ADVANTAGES	DISADVANTAGES
Cheaper source of enzymes than isolated enzymes	Sometimes have longer response times - substrate needs to diffuse through/be transported into cytoplasm;
Less sensitive to inhibition by solutes & more tolerant of pH & temperature changes leading to longer lifetimes (enzymes maintained in natural environment)	Longer recovery times (cells need to be 'reenergized')
	Less selective (contain many enzymes like tissues)

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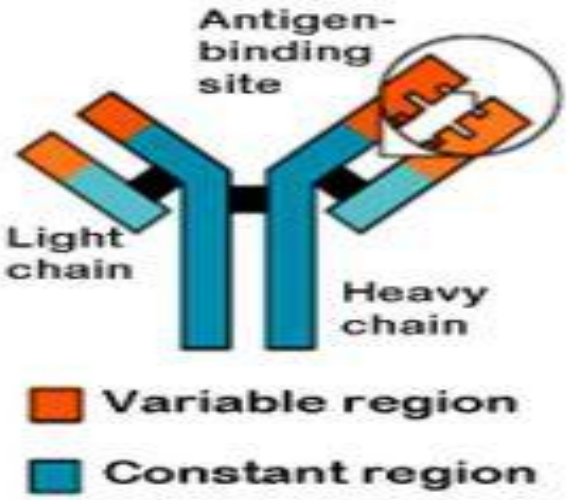
ANTIBODY/ANTIGEN

What are antibodies (Ab)?Immunoglobulins

Proteins (Y-shaped) that can bind with an invading antigen (Ag) : assist in neutralising invading/foreign particles

$$Ab + Ag \leftrightarrow Ab{\cdot}Ag$$

K_a (affinity or association constant) = 10^6



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ANTIBODY/ANTIGEN

ADVANTAGES	DISADVANTAGES
They are very selective	No catalytic effect (only a *binding* rxn occurs on contact with its Ag)
They are ultra-sensitive	Binding rxn very strong & very harsh conditions needed to reverse the rxns ⇒ biosensor can be used only once (disposable strips)
They bind very powerfully ($K_a = 10^6$)	

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NUCLEIC ACID

✓ DNA: building blocks of genetics

✓ Mechanism of biorecognition in biosensors?

Hybridization Principle : (A:T; C:G) ⇒ genosensors

✓ Operate selectively due to base-pairing characteristics :
If *sequence of bases* comprising certain part of DNA molecule known, then the *complementary sequence*, called a *probe*, can be synthesized and *labelled* with an optically detectable compound (eg., a flouresecent label).'

✓ Great potential use in identifying genetic disorders, cancers and viral infections.

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NUCLEIC ACID

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APTAMERS

✓ Aptamers - from the Latin word, *aptus*, meaning 'to fit'

✓ Molecules that have been engineered through repeated rounds of *in vitro* *selection* or equivalently, SELEX (systematic evolution of ligands by exponential enrichment) to bind to various molecular targets such as small molecules, proteins, nucleic acids, and even cells, tissues and organisms.

✓ Aptasensors are biosensors that use aptamers as their biorecognition element.

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APTAMERS

Aptamers can be classified as:

✓DNA or RNA aptamers - consist of (usually short) strands of oligonucleotides.

✓ Peptide aptamers - consist of a short variable peptide domain (10-20 amino acids), attached at both ends to a protein scaffold (eg. Thioredoxins-antioxidant protein).

✓ Aptamers offer molecular recognition properties that rival that of the commonly used biomolecule, antibodies.

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APTAMERS

Advantages:

✓ High stability

✓ Very selective and sensitive

✓ Can be engineered completely in a test tube, are readily produced by chemical synthesis, possess desirable storage properties

✓ Elicit little or no immunogenicity (does not ‘provoke’ immune response) in therapeutic applications.

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READ MORE ON APTAMERS

Strehlitz, B., Nikolaus N. and Stoltenburg, R. "Protein Detection with Aptamer Biosensors". *Sensors 2008, 8, 4296-4307.*

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