Classification of biosensors based on transducers

This lecture continues the discussion on different types of biosensors.

1 Oligonucleotide interactions

The characteristic property of complementary base pairing or hybridization between the adenine and thymine, cytosine and guanine moieties in oligonucleotide sequences can be used for detection of oligonucleotide sequences in a sample with excellent sensitivity. Such kind of sensors based on oligonucleotide sequences is commonly referred to as '*genosensor*'. The oligonucleotide sensors generally consist of an immobilized strand known as the 'probe' strand or 'sense' strand. On addition of the sample strand, if there is exact complementarity of the sequences, hybridization will occur between the probe and sample strands. This hybridization can be detected in the form of mass changes that occur when the hybridization occurs. Alternately, changes in fluorescence emission of fluorophores that are linked with the sequences can be monitored to confirm the hybridization. The specificity of the binding will depend on the length of the probe strand. If the probe strand is too short (~ 10 bp), then the probability of false positives may arise. Hence an optimum length needs to be determined for fixing the detection threshold. One of the sensors that is based on the complementary base pairing is a molecular beacon that employs a probe strand that is modified with a fluorophore at one end and a quencher on the other. In the absence of the sample strand, the probe strand will bend and the fluorescence emission of the fluorophore will be quenched. In the presence of the sample strand, hybridization will take place between the probe and sample strand resulting in stretching of the probe strand. This will cause the fluorophore to be separated from the quencher resulting in fluorescence emission. This concept is depicted in Figure 1.

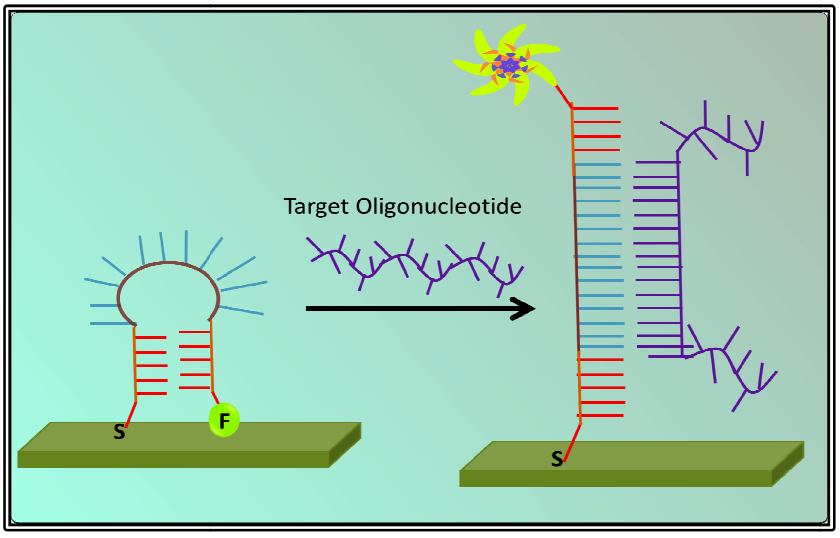

Fig 1: Principle of an oligonucleotide-based capture sensor

Similar to the complementary pairing exhibited by oligonucleotides that have been used for sensing applications, a new category of peptide nucleic acids is now used as bio-recognition elements. Peptide nucleic acids (PNA) areoligo-amides, which exhibit high affinity to their complementary oligonucleotides. Capture-based sensors employing PNA are a subject of active investigation.

2 Biomimetic receptors

Receptors that are fabricated to mimic the bioreceptors are referred to as "biomimetic receptors". Genetic engineering techniques, artificial membrane fabrication and molecular imprinting techniques have beenwidely used for fabricating biomimetic receptors. Let us consider an example of a biomimetic receptor-based sensor using artificial membrane fabrication for detectionof cholera toxins. Gangliosides (*molecules found in the ganglions of the brain and consist of glycosphingolipid with N-acetyl muraminicacid*) were incorporated into a matrix of diacetylenic lipids and were allowed to self assemble to form a liposome. It is then exposed to UV radiations that provided sufficient energy to cause photopolymerization. Figure 2 shows the reaction that occurs during the photopolymerization process.

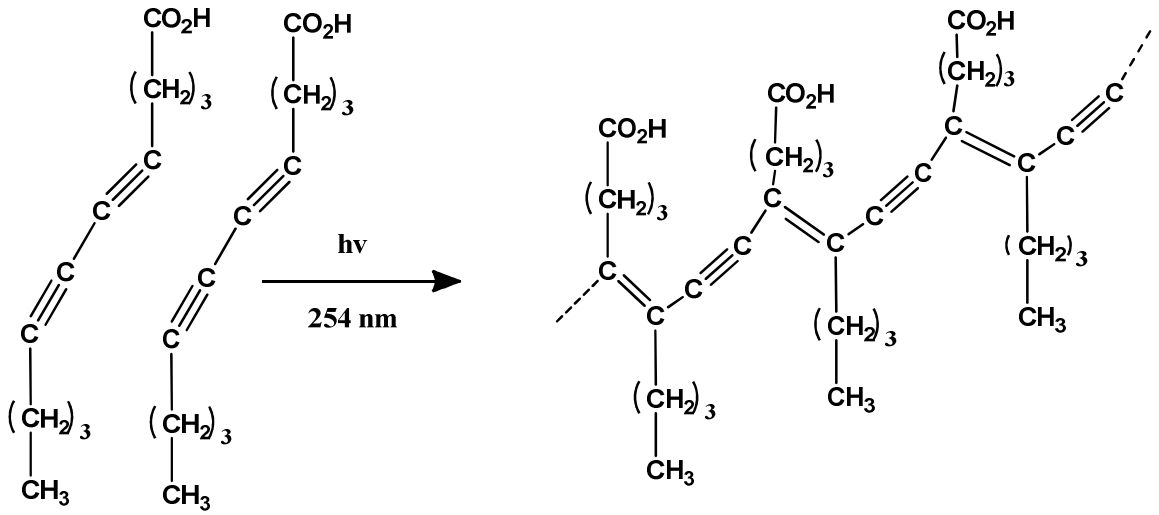

Fig 2: Photopolymerization reaction of diacetylenic lipids

It is observed that the product after photopolymerization has double bonds ('*ene'* as in alkene) and triple bonds ('*yne*' as in alkyne). This *ene-yne* system causes absorption of electromagnetic radiation in the visible region and hence the liposome appears blue or purple. This liposome system has the ability to bind with cholera toxin as natural membranes and hence forms the biomimetic receptor. Why does the cholera toxin bind to the liposomal membrane? This is because of the presence of the ganglioside! The ganglioside serves a recognition element for the cholera toxin, which is a protein that

specifically binds to gangliosides. Upon binding of the cholera toxin to the liposomal membrane, a change of colour from blue to red occurs. This colour change can be detected and quantified using spectrophotometry, as the absorption is directly proportional to the concentration of toxin present in the system. Can you guess why the colour change occurs? The binding of the protein results in reorientation of the lipid chains as well as alters the conjugation causing the colour change!

One of the key aspects of biological systems is their specificity in binding to ligands. In order to mimic this property, the concept of 'molecular imprinting' has been developed. The molecular imprinting technique has a lot of potential in detecting toxins like morphine, chemical warfare agents etc. Here, the molecule of interest (analyte) is mixed with the monomers along with the cross linkers and allowed to polymerize. After polymerization, the analyte molecules are extracted using an organic solvent thus leaving an imprint for the particular analyte. Hence when the analyte is introduced for the next time, it can simply bind to the binding site.

Did you know?....

A recent advancement in glucose sensors is contact lens based non-invasive glucose detection, which detects glucose levels from the tear fluid. The sensor uses boronic acid derivatives, which are synthetic molecules that exhibit great affinity to bind to glucose. The binding causes a change in the fluorescence emission intensity of the boronic acid derivative that can be correlated with the amount of glucose in the tear sample!

Detailed discussions on cell and tissue based sensors are given in Lecture 3 of the same module. We will now move on to another type of classification.

3 Classification based on transducers

Based on the transduction mechanism, biosensors are majorly classified into:

- Optical sensors
- Electrochemical sensors
- Mass-sensitive sensors
- Calorimetric sensors

3.1 Optical sensors

Optical sensors can convert any physical property of electromagnetic radiation into electrical signals. Optical biosensors can use a variety of spectroscopy techniques, which

includes absorption, reflectance, polarization, luminescence, fluorescence,surface enhanced plasmon resonance (SERS), refraction, dispersion, etc. The quantification is carried out based on the intensity or amplitudeof the optical signal,as a direct correlation exists between the amplitude and the concentration of the analyte.

A typical optical biosensor design will involvebinding of the analyte molecule to an optical probe resulting in change in the optical properties of the probe,which can be detected directly. Alternately, an additional 'capture agent' may be added to bind with theanalyte-probe complex. This binding in turn canlead to the formation of a coloured product that can be detected and quantified. So what are these 'capture agents'? They may be single stranded DNA specific to the complementary strand, or may be antibodies against a specific antigen. The concept of capture agents is employed in ELISA techniques that have been elaborated in Lecture 1 of the same module.

3.2 Gene chip

Introduction of more than one type of sensing probecan lead to detection of more than one type of analyte. This can be invaluable in the case of medical diagnostics as well as environmental pollution monitoring where detection of multiple analytes can be done at the same time. Such sensors have their sensing strands specific for each analyte arranged in an array. One such example of a sensor array isthe '*gene chip*'. The gene chip is a DNA microarray commonly also known as the 'DNA chip'. It is a collection of either oligonucleotides or cDNA-based fragments (referred as probe sequences) attached to a solid support. (*A cDNA or complementary DNA is complementary to the mRNA sequence and does not contain the introns. It in fact contains the complementary gene sequence leading to the protein!*).The density of the oligonucleotide sequence in each spot as well as the base pair length are factors that will influence the sensor performance. Typically, each spot is composed of approximately 40 oligonucleotides each with a base pair length of about 25.A matching control spot with similar density and base pair length but with one central base changed is also available. Spotting of the oligonucleotide sequences has been achieved through many techniques such as atomic force microscopy, ink jetting, laser-assisted bioprinting etc.

It is necessary to know the sequence that is being detected in order to design the probe strands. The length of the probe strand must be carefully chosen. One major challenge involved in the DNA microarray is the need of different permutations for each gene in order to avoid erroneous false positives due to short probe sequences. In the case of a cDNA microarray, the cDNA-sized fragmentsare usually produced by polymerase chain reaction. The cDNA are long fragments that uniquely identify a specific gene. The advantages of such microarrays are:

- Small size (*A 1.3 x 1.3 cm slide can pack up to 6000 genes!*)

- High sensitivity
- High throughput (*Can detect thousands of different sequences at the same time!*)
- Small analyte volume

This microarray technology is widely used in clinics, where mRNA is isolated from the cell samples obtained from patients,labeled with a fluorescent probe and applied to the chip containing the probe strands. Hybridization takes place between the complementary strands, if present. Upon hybridization, the fluorescence intensity is detected and quantified by scanning the glass slide containing the microarray. Gene chips have the ability to detect mRNA present at <1 molecule in 100,000. Entire genomes can be now introduced into a single chip! Figure 3 shows the principle of a gene chip for detection of a pathogen.

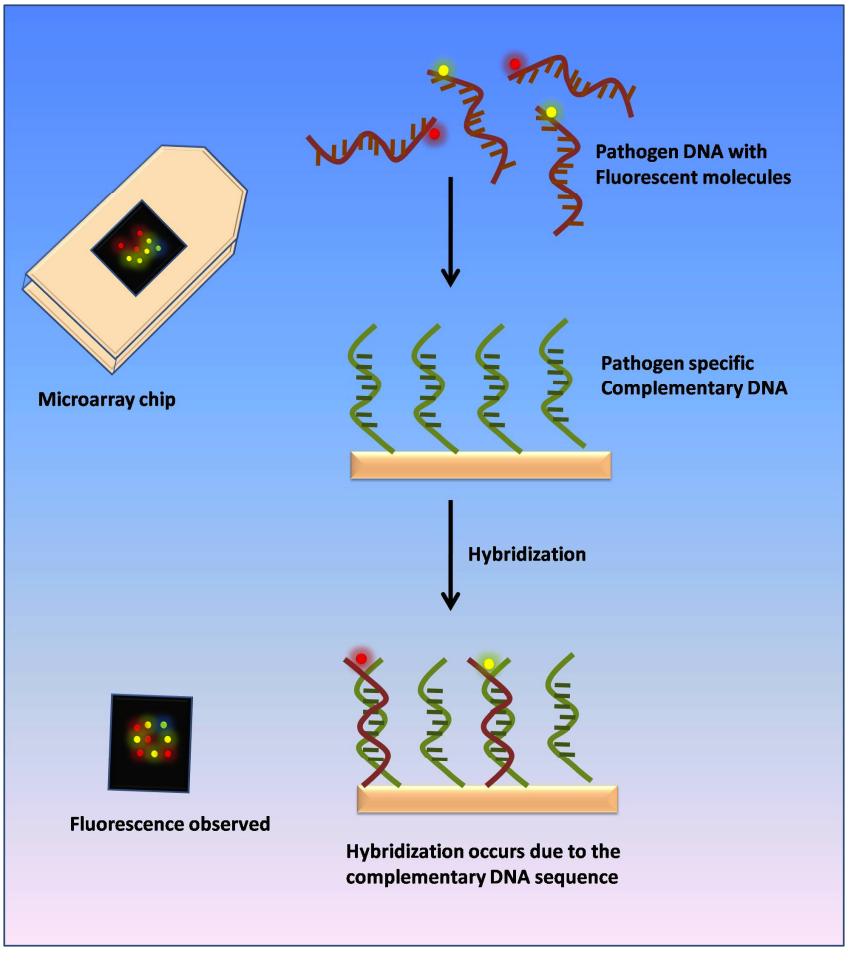

Fig 3: Schematic representation of a gene chip for detection of pathogens

3.3 Optical barcoding

Optical barcoding is a recently evolved technology making use of quantum dot–loaded microsphere capture reagents. Quantum dots are semi-conductor nanoparticles that

exhibit size-dependent fluorescence emission. (*More detailed discussions on quantum dots is given in Module 12*). Taking into account of the disadvantage of fluorescent probes as they are susceptible to photo-bleaching, researchers have come up with the use of quantum dot technology. The most important advantage of quantum dot is its size dependent luminescence and narrow emission bands from a common excitation wavelength. Moreover, it is also stable against photobleaching. Optical barcoding involves loading the polymeric microspheres with different amounts of several colors of quantum dots to obtain a unique fluorescence signature. Six colors at 10 possible intensities allows for $>10^6$ possible codes. Capture molecule on surface of beads grabs the labeled analyte and it can be detected.

3.4 Optic fiber sensor

One of the emerging trends in clinical diagnosis is to detect an anomaly even before the onset of symptoms. Any disease or disorder is initiated at a molecular level, which later is amplified into a cellular level and then spreads to multiple cells. If a considerable amount of the tissue/organ is impaired, then the individual starts manifesting the symptoms. In many cases, the onset of symptoms signifies an advanced stage of the disease, which makes it difficult to treat thereby reducing the survival rate. Hence, diagnosis at the molecular level has a major implication on the success of the treatment. This aspect has been realized with the advent of nanotechnology where analyte detection in the zeptomolar range (10^{-21} M) has been reported. This means that a few hundred molecules of the analyte can be detected!

An example of a super ultra-sensitive sensor employs a fiber optic probe whose tip is etched to obtain a tapered tip about 40 nm wide at its narrowest point. The nano-dimensions of the tip and the tapered geometry facilitate the penetration of the probe into a cell. On insertion into the cell, the membrane bilayer self-seals around the fiber optic probe and hence no permanent damage to the cell membrane occurs. Why is a fiber optic probe used? An optic fiber is one in which the intensity of the light radiation is transmitted without any loss and hence can be used for high sensitivity optical detection without any loss in signal intensity. The tip of the optic fiber is chemically functionalized with an antibody againstbenzopyrenetetrol. The chemical functionalization is essential because if the antibody were to be physisorbed on the probe, the risk of the antibody being desorbed will be high. Benzopyrenetetrol is the metabolic product of benzopyrene, a chemical carcinogen. How doesbenzopyrene gain entry into the biological system? Benzopyrene is a polycyclic aromatic hydrocarbon that is found in automobile emissions and smoke emissions due to burning of wood. Yet another common source of benzopyrene is grilled and/or deep fried food items!

 (*OOPS!!! Most of my favourite fast food items might be detrimental to my health!!!*)

Once the benzopyrene enters the system, it can undergo a series of enzyme-catalyzed reactions resulting in the formation of benzopyrenetetrol. The reactions leading to the formation of benzopyrenetetrol from benzopyreneis shown in Figure 4.

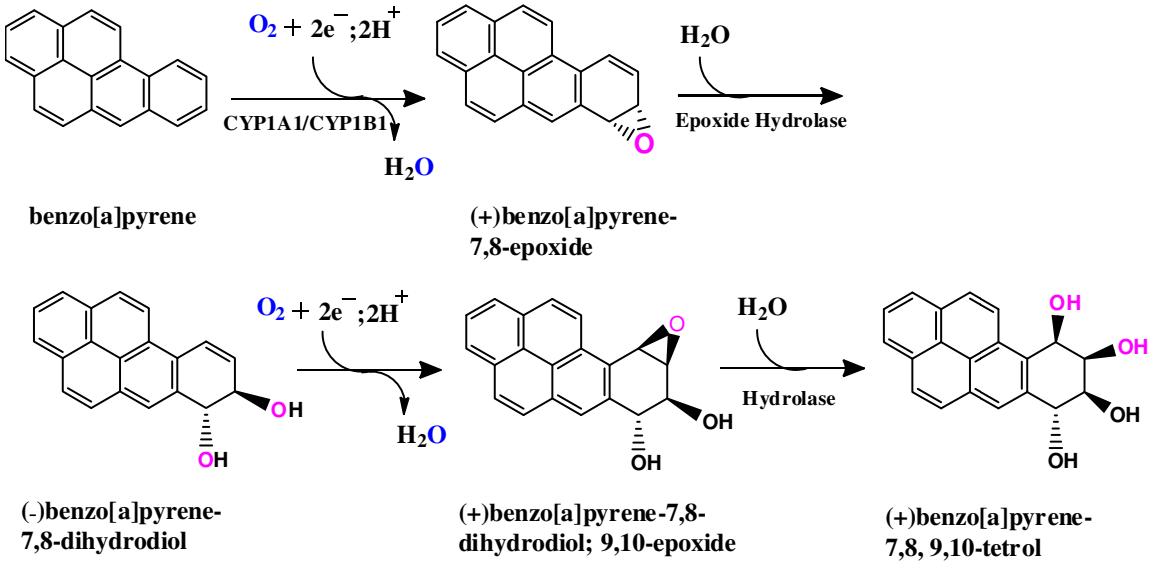

Fig 4: Reactions leading to the formation of benzopyrenetetrol from benzopyrene

The tetrol derivative can interact with DNA, which in turn can transform the normal cell into a cancerous cell. This is because the tetrol molecule has four hydroxyl groups that can interact with the sugar and phosphate groups of the DNA.The presence of benzopyrenetetrol in the cell therefore indicates a potential risk of cancer development. Thus, quantification of this analyte can be a measure of the risk of developing cancer in an individual.The probe when inserted into the cell exposes the antibody to the cytosol. If benzopyrenetetrol is present in the cell it will bind with the antibody, forming an immune complex, which can be detected from the unique fluorescence emission wavelength of the immune complex.

4 Reference

Biosensors and biochips: advances in biological and medical diagnosis,Tuan vo-Dinh, Drian Cullum, Freselaus J Analytical Chemistry, 2000, 366, 540-551.