MOLECULAR RECOGNITION

BIOANALITICAL METHODS

BIOSENSORS

• Molecular Recognition
  – Bioreceptor-Analyte interaction
• Chemical and/or Physical Signal Generation
  – produced by molecular recognition
• Transducer
  – detects response directly and converts it to a signal that can be amplified, stored or displayed
  Or
  – detects the signal provided by a chemical mediator
• Chemical Mediator
  – interacts with the molecular recognition system
  – this interaction produces a secondary signal that is transduced
Bioreceptors (Probes)

- **Enzymes**
  - Catalyze reactions
  - Are highly specific
  - Are self-regenerating
  - Are easy to incorporate in devices while preserving their integrity

- **Antibodies: immunosensors**
  - Specific
  - Single use or require washing after use
  - Easy to incorporate in devices

- **Others**
  - Compounds, cells, microorganelles that can participate in molecular recognition of the analyte of interest

Transducers

- Type of transducer depends on type of response
  - Generation of protons or hydroxide ions: pH indicator and optical device or pH change sensor
  - Redox reactions: amperometric, potentiometric or conductometric transducers
  - Exothermic or endothermic reactions: thermal transducers
Operating Principle of a Biosensor

Blood Glucose Biosensor

- Analyte: glucose
- Bioreceptor: glucose oxidase
- Amperometric detection
- Molecular recognition response: Glucose oxidase (GOx) oxidizes glucose to gluconic acid. GOx is reduced.
- Chemical mediator: Ferrocenium/Ferrocene
Operating Principle of Amperometric Detection of Glucose

- Mediation reaction: reduced GOx is oxidized by a chemical mediator ferrocenium.
  - Reduction potential of mediator must be higher than reduction potential of GOx

- The reduced mediator (ferrocene) is oxidized at an electrode generating a current
  - Electrode potential is such that background current is minimized (other components in the blood are not oxidized)

Molecular Mediator in the Amperometric Glucose Sensor

- Ferrocenium/ferrocene
• Strategies for the immobilisation of enzymes
  – physically adsorbed
  – covalently attached
  – entrapped onto the transducer
• Performance characteristics of interest for a sensor
  – working range
  – Sensitivity
  – limit of detection
  – selectivity/specificity
  – response time
  – stability.

An Array Biosensor-Affinity based biosensor without a label using Surface Plasmon Resonance(SPR)-based Detector

• Exploring “one-shot” kinetics and small molecule analysis using the ProteOn XPR36 array biosensor
• Tsafrir Bravman a, Vered Bronner a, Kobi Lavie a, Ariel Notcovich, Giuseppe A. Papalia, David G. Myszka
• Kinetics and binding affinity measurements
  – What is SPR?
  – Describe how the ProteOn XPR36 operates to provide kinetic and binding affinity data
  – Summarize results of the paper
  – Critical evaluation of method/ results
Surface Plasmon Resonance (SPR)-based biosensor

- High-resolution characterization of antibody fragment/antigen interactions using Biacore T100
- Giuseppe A. Papalia, Mark Baer, Kenneth Luehrsen, Helena Nordin, Peter Flynn, David G. Myszka
- Describe the signal transduction system
- List the advantages of SPR

Sensors 2004, 4, 71-83

- Supermolecular Interaction of Ferrocenium with Yeast DNA and Application in Electrochemical Sensing for Hybridization Recognition of Yeast DNA
- Huangxian Ju*, Baofen Ye and Jiayin Gu
- Describe the molecular recognition and signal transduction processes
DNA Binding Arrays

• Molecular recognition
  – Matching of complementary oligonucleotides
• Probes: array of oligonucleotides immobilized on a glass substrate (slide) with separate spots
• Targets: single stranded DNA fragments with colored/fluorescent labels
• Array is treated with sample of DNA digest
• Spots with a complement will hybridize with ssDNA fragments
• Hybridization at 60°C for 12 to 16 hours
• Hybridized spots take the color of the label
• The Slide is washed, then analyzed
• The array pattern of labeled and unlabeled is determined by the DNA sequence, thus is a fingerprint of the DNA

Principle of Molecular Recognition in DNA Array Reactions

Fig. 5.21. The principle of molecular recognition in DNA array reactions.
DNA Arrays

- Macro arrays
  - Spot size 300um
  - Probe oligonucleotide dropped onto substrate
  - Custom-made with 1000 to 10,000 different spots per glass

- Microarrays
  - Spot size 20 – 50 µm
  - Oligonucleotides directly synthesized on chip using photolithography (light activated reactions)
  - Oligomer size: 25 nucleotides
  - Whole GeneChip™ by Affymetrix
    - >400,000 different oligonucleotide probes

Fig. 5.22. A DNA array is an orderly arrangement of immobilised oligonucleotides on a glass slide, each grey spot represents a different oligonucleotide. When reacted with labelled DNA samples, they hybridise with only certain spots on the array, i.e. those containing a matching oligonucleotide sequence. This results in a characteristic pattern, a fingerprint, of coloured and uncoloured spots.
DNA array: use of nylon for immobilization and fluorescent labels for detection

- Use of nylon DNA binding surfaces for hybridization between immobilized DNA probes and fluorescently labeled DNA Targets
- Sarah Stephens, Elizabeth Bull, David Cullen, Phillip Warner, Jeff Kane and Peter Ball
- Email

DNA Identification by Pyrosequencing

- Response to molecular recognition is a chemiluminescent enzymatic reaction
- Used for sequencing of short segments (50-60 base) ssDNA
- Applications
  - Typing of viruses and bacteria
  - Sequencing of disease associated genes
  - Determination of Single Nucleotide Polymorphism (SNPs)
DNA Identification by Pyrosequencing

- ssDNA immobilized onto surface
- Primer is hybridized to the ssDNA
- Addition of four enzymes and two substrates
  - DNA polymerase
  - ATP sulfuryrase and Adenosine 5’phosphosulfate (APS)
  - Luciferase and luciferin
  - Apyrase
- Addition of one of the four dNTPs

If dNTP added is a complement of the nucleotide following those bound to the primer, DNA polymerase will catalyse its incorporation. Pyrophosphate($P_2O_7$)$^{4-}$, is released (PPI).

- (Oligonucleotide)$^n$ + dNTP + DNA poly $\rightarrow$ (Oligonucleotide)$^{n+1}$ + PPI
- PPI reacts with APS to produce ATP via ATP Sulfuryrase catalysis
  - APS + PPI + ATP sulfuryase $\rightarrow$ ATP + sulfate
- Luciferase (Icfrase) catalyses the reaction of ATP with the substrate luciferin (Icfr) producing oxyluciferin and visible light
  - ATP + Icfr + Icfrase $\rightarrow$ AMP + phosphat + Oxylfr+ hv
- Light intensity is directly proportional to the number of dNTP incorporated (up to 5 or six bases)
DNA Identification by Pyrosequencing

• Excess dNTP and ATP are degraded by the enzyme apyrerase to their corresponding mono- and diphosphates
  - $\text{dNTP} + \text{Apyrase} \rightarrow \text{dNDP} + \text{dNMP} + (\text{PO}_4)^{3-}$
  - $\text{ATP} + \text{Apyrase} \rightarrow \text{ADP} + \text{AMP} + (\text{PO}_4)^{3-}$
• Add next dNTP etc.

Fig. 5.30. Pyrogram™ obtained from sequencing the immobilised target DNA.