DNA based affinity biosensors
Outline

- Why DNA?
- DNA biosensors
  - PCR
  - Example of DNA biosensors
  - DNA Microarray/Chip
- Molecular Beacons
  - What is a molecular beacon
  - Use of molecular beacons as sensors
- Aptamer
  - What is an aptamer
  - Selex
  - Examples of aptasensors
- PCR based sensors
Of what we will talk?

**Genosensors:** Use *Nucleic acid* as recognition element for the recognition of DNA/RNA.

**Aptasensor:** Use *aptamers* (DNA or RNA) as recognition elements for the detection of not nucleic acid targets.
Just a small reminder

**Genome:** The totality of the genetical information of a cell/organism.

**Gene:** Segment of the DNA that code the production of a protein.

**Nucleic Acid:** Basic constituent of the DNA.
The unique affinity/recognition properties of the oligonucleotides (DNA) allow the development of biosensors that can provide specific qualitative and semi-quantitative analytical information.
**Hybridisation.** Process of creating a double stranded nucleic acid chain (DNA, RNA or mixed segment) starting from single stranded chains.

DNA denaturation is reversible by keeping the two single stands of DNA for a prolonged period at 65° C = 149° F. This process is called **DNA renaturation** or hybridization.

Similar hybridisation reactions can occur between any single stranded nucleic acid chain: DNA/DNA, RNA/RNA, DNA/RNA.

These hybridisation reactions can be used to detect and characterise nucleotide sequences using a particular nucleotide sequence as a probe.
Parameters influencing the hybridisation

**Strand length**

The longer the probe the more stable the duplex

**Base Composition**

The % G:C base pairs are more stable than A:T

**Chemical environment**

The concentration of Na$^+$ ions stabilise the DNA/DNA duplex.

Chemical denaturants (formamide or urea) destabilise the DNA/DNA duplex.

**Reassociation kinetics:**

When double stranded DNA is denatured by heat the speed at which the strands form double stranded DNA is due to the **starting concentration** of DNA. If there is a high concentration of complementary DNA then the time required will be reduced.
Polymerase Chain Reaction: The PCR method - a copying machine for DNA molecules - DNA molecules can be mass-produced from incredibly small amounts of material with PCR. Kary Mullis' discovery allows the chemist to mimic the cell's own natural DNA replication process in a test tube. It has now become much easier to characterise and compare the genetic material from different individuals and organisms.
Some key concepts to remember

**Importance:** In vitro replication and functionalisation of DNA, revolutionising the field of molecular biology.

**Key to Kary Mullis’ observation:** Extremophyles (organisms that live in extreme environments – in this case high temperatures) replicate DNA efficiently at high temperatures!!!!!!

**Kary Mullis’ invention:** Use enzymes from extremophyles in replication!!!
How PCR works

The hybridisation of a short oligonucleotide (primer) to a longer DNA to start the DNA synthesis is also the basis of the polymerase chain reaction (PCR).

Figure 8–39 part 1 of 3. Molecular Biology of the Cell, 4th Edition.
DNA Chip/Microarray
Micro-array Technology

- Measurement of the **expression levels** of thousands of genes **simultaneously**
  - Sequencing of the genome (human and laboratory animals)

- To probe the **genome** of an organism to identify specific sequences.
What is a micro-array and for what it is used?

- **Small, solid support** (glass, silicon chip or nylon membrane) on which the **sequences** from thousands (tens of thousands) of **genes** are **attached** at **fixed locations**.
- The DNA is **printed, spotted** or **synthesized** directly onto the support.
- The spots can be **DNA, cDNA** or **oligonucleotides**.
- **Compare gene expression** in **two different** cell types or tissue samples.
- Examine **expressions** in a single sample on a genome-wide scale (GENOMICS).
- **Infer new gene functions, diagnostic tools** – e.g. in cancer provides a molecular view.
What information can we get and why they are important?

- What genes are Present/Absent in a cell?
- What genes are Present/Absent in the experiment vs. control?
- Which genes have increased/decreased expression in experiment vs. control?
- Which genes have biological significance?

- Just because we sequenced a genome doesn’t mean we know everything about the genes. Thousands of genes remain without an assigned function.
- Patterns/clusters of expression are more predictive than looking at one or two prognostic markers – can figure out new pathways.
Steps of a DNA Micro-array Experiment

1. **Manufacturing** of the micro-array.

2. Experimental design and choice of reference: **what to compare to what?**

3. Target preparation (**labeling**) and **hybridization**.

4. Image **acquisition** (scanning) and **quantification** (signal intensity to numbers).

5. **Database building**, filtering and normalisation.

6. **Statistical analysis** and **data mining**.
Array preparation: Spotting technology

- **Steel spotting pin**
- **Chemically modified slides**
- **384 well source plate**

1 nanolitre spots
90-120 um diameter
Direct synthesis of oligonucleotide probes onto chip surface (Affymetrix)

Probe arrays are manufactured in-situ by light-directed chemical synthesis process which enables the synthesis of hundreds of thousands of discrete compounds in precise locations.

http://www.affymetrix.com/corporate/outreach/educator.affx
## Advantages and disadvantages

<table>
<thead>
<tr>
<th>Spotted Micro-array</th>
<th>Affymetrix GeneChip approach</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Flexible and cheaper</strong></td>
<td>Good for whole genome expression analysis where genome of that organism has been sequenced—&quot;Off the rack&quot;</td>
</tr>
<tr>
<td>Allows study of genes not yet sequenced (spotted ESTs can be used to discover new genes and their functions).</td>
<td>High quality with little variability between slides</td>
</tr>
<tr>
<td>Tailored</td>
<td>Gives a measure of absolute expression of genes</td>
</tr>
<tr>
<td>Variability in spot quality from slide to slide</td>
<td>Commercially available</td>
</tr>
<tr>
<td>Provide information only on relative gene expressions between cells or tissue samples</td>
<td>Maximum 500,000 features per array</td>
</tr>
<tr>
<td>Homemade</td>
<td>Less variability</td>
</tr>
<tr>
<td>Maximum 24,000 features per array</td>
<td>More expensive yet less flexible</td>
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</tbody>
</table>
cDNA Micro-array

- Both a sample and a control are used as targets.
- mRNA is extracted from sample and control and converted to cDNA via reverse transcriptase.
- cDNA is amplified via PCR.
- DNA probe is deposited onto substrate.
- Target DNA is fluorescently tagged, (Cy3 control, Cy5 Target) added to the array and hybridisation does or does not take place.
- If no hybridisation, no color results.
- If sample (red) DNA hybridises and control does not, red color results.
- If control (green) DNA only hybridises, green color results.
- If both hybridise equally, yellow color results.
- Color intensity depicts the level of mRNA expression.
Microarray preparation

Control Cell

Experimental Cell

mRNA extracted from cell

Reverse transcription, fluorescently labeled with Cy3 (Green) and Cy5 (Red)

Combine equal amount and hybridize onto microarray

cDNA microarray

Scan
How to understand cDNA chip

Red: High expression in target labelled with cyanine 5 dye.
Green: High expression in target labelled with cyanine 3 dye.
Yellow: Similar expression in both target samples.
Oligonucleotides micro-array

- Similar to the cDNA array except that only sample is hybridised (no control) and the target is genomic DNA.

- Different fluorescent dyes are not needed.

Often biotin is introduced in the amplified target DNA to allow labelling of it with Streptavidin modified with fluorophores.
Oligonucleotide micro-arrays: transduction

Array

Hybridised Array

Biotin modified target

Streptavidin-phycoerythrin conjugate
Genotyping using enzymatic label

1. Target allele hybridization
2. DNA-HRP conjugate hybridization
3. Amperometric detection

Absorbance / a.u.

- DQA0501 probe
- DQA0301 probe
- DQB02 probe
- DQA0201 probe

Electrochemical

Amperometric detection

Colorimetric

OR
Example of commercially available DNA chips (Affimatrix)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Genome Arrays</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. subtilis</td>
<td>Barley Genome Array</td>
</tr>
<tr>
<td></td>
<td>Bovine Genome Array</td>
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<tr>
<td></td>
<td>C. elegans Genome Array</td>
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<td></td>
<td>Canine Genome Array</td>
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<td>Chicken Genome Array</td>
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<td>Drosophila Genome Arrays</td>
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<td>E. coli Genome Arrays</td>
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<td>Human Genome Arrays</td>
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<td>Maize Genome Array</td>
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<td></td>
<td>Mouse Genome Arrays</td>
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<td></td>
<td>P. aeruginosa Genome Array</td>
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<tr>
<td>Plasmodium/Anopheles</td>
<td>Porcine Genome Array</td>
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<td></td>
<td>Rat Genome Arrays</td>
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<td></td>
<td>Rice Genome Array</td>
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<td></td>
<td>Soybean Genome Array</td>
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<tr>
<td></td>
<td>Sugar Cane Genome Array</td>
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<tr>
<td></td>
<td>Vitis vinifera (Grape) Array</td>
</tr>
<tr>
<td></td>
<td>Wheat Genome Array</td>
</tr>
<tr>
<td></td>
<td>Xenopus laevis Genome Array</td>
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<tr>
<td></td>
<td>Yeast Genome Arrays</td>
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<td></td>
<td>Zebrafish Genome Array</td>
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<td></td>
<td>Arabidopsis Genome Arrays</td>
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</tbody>
</table>
Molecular Beacons
Transduction

Electrochemical MB

Fluorescence MB

Au electrode

Molecular Beacon

Target

Hybrid

Fluorophore

Quencher
Fluorescence MB

Gold-Nanoparticle-Based Multicolor Nanobeacons for Sequence-Specific DNA Analysis

Shiping Song, Zhiqiang Liang, Juan Zhang, Lihua Wang, Genxi Li,* and Chunhai Fan*

Anal. Chem. 2009, 81, 8826–8830
Electrochemical MB

Hybridisation with specific (Mut) and unspecific (Wt) target

Electrochemical molecular beacon DNA biosensor for the detection and discrimination of the DF508 cystic fibrosis mutation

Hany Nasef*, Valerio Beni*, Ciara K. O'Sullivan ab,*

*Departamento de Ingeniería Química, Universitat Rovira i Virgili, Avinguda Països Catalans, 26, 43007 Tarragona, Spain
bICREA, Parc Científic de Barcelona 23, 08010 Barcelona, Spain

Journal of Electroanalytical Chemistry 662 (2011) 322–327
Other possible DNA assay formats
Nano-particles based colorimetric detection of DNA

Research Article

Gold Nanoparticle Sensor for the Visual Detection of Pork Adulteration in Meatball Formulation


1 Institute of Nano Electronic Engineering, Universiti Malaysia Perlis, Kangar, Perlis 01000, Malaysia
2 Halal Products Research Institute, Universiti Putra Malaysia, UPM Serdang, Selangor 43400, Malaysia
3 Department of Preclinical Sciences, Faculty of Veterinary Medicine, Universiti Putra Malaysia, UPM Serdang, Selangor 43400, Malaysia

Journal of Nanomaterials
Volume 2012, Article ID 103047, 7 pages
doi:10.1155/2012/103047

Figure 4: Determination of LOD for pork in ready-to-eat beef meatballs. In the inset, vials (a)–(e) demonstrate the color of gold nanoparticles in 1% (a), 3% (b), 5% (c), 10% (d), and 15% (e) pork DNA extracted from processed pork-beef meatballs. The corresponding absorption spectra are shown with alphabetical labels. The LOD is shown to be 10% (4 μg/mL) of swine DNA in mixed meatball preparation (vial (d) and spectrum (d)).
Surface plasmon resonance detection of oligonucleotide sequences of the rpoB genes of *Mycobacterium tuberculosis*

A. Rachlovský, S. Patskovský, A. Soldatkin, M. Meunier

*Institute of Molecular Biology and Genetics of National Academy of Sciences of Ukraine, 150 Academicova Zelivska Street, Kyiv 03143, Ukraine*

*Engineering Physics Department, Laser Processing and Plasma-Energetic Laboratories, École Polytechnique de Montréal, C. P. 6079, succ. Centre-Ville, Montréal (Québec), Canada P3C 3A7*

Talanta 85 (2011) 2094–2099

Concentration response curve
QCM for DNA hybridisation monitoring

Hybridisation-Regeneration Cycle

![Hybridisation-Regeneration Cycle Diagram](image)

**Graph Details:**
- **Y-Axis:** Frequency (Hz)
- **X-Axis:** Time (sec)
- Stages:
  - Buffer
  - Sample
  - Washing
  - Regeneration
  - Buffer
Real time PCR

Amplification in the presence of fluorescence molecular beacon (probe).

If target is present increase in Fluorescence response.
Aptamers are proposed as **alternatives to antibodies** as biorecognition elements in analytical devices with ever increasing frequency.

Aptamers are **nucleic acid** (DNA or RNA) **artificial ligands** that **can be generated** against amino acids, drugs, proteins and other molecules. Name derives form the Latin word ‘aptus’, which means ‘to fit’

They are **isolated from complex libraries** of synthetic nucleic acids by an **iterative process of adsorption, recovery and amplification**, called **systematic evolution of ligands by exponential enrichment (SELEX)**.

**SELEX**

- **Evolution studies**
  - Cloning and sequencing
  - Individual aptamer

- **Amplification**
  - Random oligonucleotide library
  - 22f, 49n, 23r

- **Binding**
  - Target

- **Partitioning**
  - Unbound sequences

- **15 rounds**
Automated ‘robot’ SELEX
## Advantages of Aptamers

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Aptamers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limitations against target representing constituents of the body and toxic substances</td>
<td>Toxins as well as molecules that do not elicit good immune response can be used to generate high affinity aptamers</td>
</tr>
<tr>
<td>Kinetic parameters of Ab-Ag interactions cannot be changed on demand</td>
<td>Kinetic parameters such as on/off rates can be changed on demand</td>
</tr>
<tr>
<td>Antibodies have limited shelf life and are sensitive to temperature and may undergo denaturation</td>
<td>Denaturated aptamers can be regenerated within minutes, aptamers are stable to long-term storage and can be transported at ambient temperature</td>
</tr>
<tr>
<td>Identification of antibodies that recognize targets under conditions other than physiological is not feasible</td>
<td>Selection conditions can be manipulated to obtain aptamers with properties desirable for <em>in vitro</em> assays</td>
</tr>
<tr>
<td>Antibodies often suffer from batch to batch variation</td>
<td>Aptamers are produced by chemical synthesis resulting in little or no batch to batch variation</td>
</tr>
<tr>
<td>Requires the use of animals</td>
<td>Aptamer generation does not require animals</td>
</tr>
<tr>
<td>Labelling of antibodies can cause loss in affinity</td>
<td>Reporter molecules can be adjusted to aptamers at precise locations not involved in binding</td>
</tr>
</tbody>
</table>
Examples of molecules for which aptamers have been selected in vitro:

Examples of possible targets

<table>
<thead>
<tr>
<th>Target/ligand</th>
<th>Recognition element</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>Adenosine aptamer in duplex</td>
</tr>
<tr>
<td>Adenosine</td>
<td>Adenosine aptamer in partial duplex</td>
</tr>
<tr>
<td>Adenosine</td>
<td>Adenosine aptamer in partial duplex</td>
</tr>
<tr>
<td>Adenosine or thrombin</td>
<td>Adenosine or thrombin aptamer in duplex</td>
</tr>
<tr>
<td>Thrombin</td>
<td>Thrombin aptamer in duplex</td>
</tr>
<tr>
<td>Thrombin</td>
<td>Single-stranded thrombin aptamer</td>
</tr>
<tr>
<td>Cocaine</td>
<td>Single-stranded cocaine aptamer</td>
</tr>
</tbody>
</table>

Aptamer for detection of cancer

Arginine
Dopamine
Membrane receptors
Whole viruses
Reverse transcriptase of HIV

X. Zhu et al. / Analytica Chimica Acta 764 (2013) 59–63

Importance of intra-molecular interaction on recognition with aptamers.

The majority of aptamer structures result from intramolecular base pairing to produce loops or bulges, forming structures such as the hairpin, the pseudo knot, and the stem-loop/bulge. A different type of structural motif is the G-quartet, also known as “quadruplex”, “tetraplex” or “G4” DNA.

Aptamer transduction
Example of Electrochemical Aptasensor

Comparing analytical parameters, best results obtained with direct detection of thrombin reaction product.

Different approaches for the detection of thrombin by an electrochemical aptamer-based assay coupled to magnetic beads.

S. Centi*, G. Messina, S. Tombelli, T. Pachetti, M. Mascini

S. Centi et al. / Biosensors and Bioelectronics 23 (2008) 1602–1609
Enzyme label based detection

Quite a complex sandwich system for detection of IgE. Enzyme label facilitates lower detection limits of 0.02nM using linear sweep voltammetry. Highly selective towards IgE.

Electrochemical immunosensor with aptamer-based enzymatic amplification
Kejun Feng, Yan Kang, Jing-jin Zhao, Ya-Li Liu, Jian-Hui Jiang*, Guo-Li Shen, Ru-Qin Yu*

Analytical Biochemistry 378 (2008) 38–42
Structural modulated detection

A Target-Responsive Electrochemical Aptamer Switch (TREAS) for Reagentless Detection of Nanomolar ATP
Xiaolei Zuo,†‡ Shiping Song,‡ Jiong Zhang,‡‡ Dun Pan,‡ Lihua Wang,‡ and Chunhai Fan†‡
J. AM. CHEM. SOC. 2007, 129, 1042–1043

Scheme 1. The TREAS Strategy for ATP Detection

In the initial state, the ATP aptamer forms a duplex and ferrocene is distal to the electrode surface (et OFF; et stands for electron transfer); after reaction with ATP, the aptamer forms a tertiary structure with ATP and liberates its complementary strand. Ferrocene is proximal to the electrode surface (et ON) in this state.

Label-Free Electronic Detection of Thrombin in Blood Serum by Using an Aptamer-Based Sensor

Yi Xiao, Arica A. Lubin, Alan J. Heeger, and Kevin W. Plaxco*

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1.0
1.5
I, µA

1 mM ATP

1 mM CTP/GTP/UTP

E, V

120
110
100
90
80
70
60
50
40
30
20
10
0

buffer

regenerated

buffer + thrombin

E / V

---
Fluorescence apta-beacon
Detection of hardness in water using quadruplex formation of G-rich aptamer. Potassium interference is eliminated by heating.

Potential interfering effect of other ions found in water studied.
DNA can find application in biosensor technology for the detection of nucleic acids (DNA probes) or for the detection of non-nucleic acid targets (aptamers).

PCR is an essential tool in DNA biosensing.

We saw what are DNA-microarrays (cDNA and oligonucleotide) and how they can be used.

We defined what are aptamers and how these can be used in biosensing.

We saw how different transduction approaches (fluorescence, electrochemical, mass based, colorimetric, SPR.....) can be adapted to perform geno/apta sensing.