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New method for nucleotides diagnosis

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OUTLINE

• Introduction
• Types of nucleotides diagnosis
• Principles of Nucleic acid hybridization
• Types of DNA Based Biosensors
• Electrochemical DNA Biosensor
• Immobilization of DNA Probe onto Transducer Surface
• Effective factors on hybridization
• Diagnostic methods for hybridization event
• Conclusions
DNA structures—double helix (complementary)

- 4 bases:
  - Adenine (A), Guanine (G),
  - Thymine (T), and Cytosine (C)
- sugar (deoxyribose)
- phosphate group
DNA STABILITY

- Hydrogen bonding between base pairs
- Stacking interaction between bases along axis of double-helix
- Size and base content and sequence
TYPE OF OLIGONUCLEOTIDE DIAGNOSIS

- Method based on Sequencing
- Method based on Amplification
- Method based on Hybridization
---rennealing b/w the ssDNAs from different sources

- **Perfect match** ---stable dsDNA, strong hybridization
- **One or more base mismatches** ----weak hybridization
Principles of Nucleic acid hybridization

- ssDNA (Probe)
- (Target Sequence)
- (Hybridization)
- (Stable dsDNA)
**Sensor:** A sensor is a device that produces a measurable signal in response to a stimulus.

**Transducer:** A transducer is a device that converts one form on energy into another.
## Types of DNA based biosensors

- Optical, Electrochemical and Piezoelectric

### Table 1: Types of DNA biosensors.

<table>
<thead>
<tr>
<th>Type</th>
<th>Biological Element</th>
<th>Transducer</th>
<th>Reference</th>
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</thead>
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<tr>
<td><strong>Optical</strong></td>
<td>DNA</td>
<td>Optical fiber</td>
<td>Piunno <em>et al.</em> [26]</td>
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<tr>
<td>Fiber optics</td>
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<td></td>
<td>Piunno <em>et al.</em> [27]</td>
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<td></td>
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<td>Hirschfeld [28]</td>
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<td>Surface plasmon resonance</td>
<td>Resonant mirror</td>
<td>Watts <em>et al.</em> [29]</td>
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<td>Biomolecular interaction analysis</td>
<td>BIAcore</td>
<td>Nilsson <em>et al.</em> [30]</td>
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<td>Wood [31]</td>
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<td>Raman spectroscopy</td>
<td>SERG probes</td>
<td>Vo-Dinh <em>et al.</em> [32]</td>
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<tr>
<td><strong>Electrochemical</strong></td>
<td>DNA</td>
<td>Carbon paste electrodes</td>
<td>Millan <em>et al.</em> [33–35]</td>
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<tr>
<td><strong>Piezoelectric</strong></td>
<td>DNA</td>
<td>Crystals</td>
<td>Campbell <em>et al.</em> [36]</td>
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<td>Frequency</td>
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<td>Okayahara <em>et al.</em> [37]</td>
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<td></td>
<td>Crystals</td>
<td>Su <em>et al.</em> [25,38,39]</td>
</tr>
<tr>
<td>Acoustics</td>
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</tbody>
</table>
Basic characteristics of a biosensor

1. **LINEARITY**
   Linearity of the sensor should be high for the detection of high substrate concentration.

2. **SENSITIVITY**
   Value of the electrode response per substrate concentration.

3. **SELECTIVITY**
   Chemicals Interference must be minimised for obtaining the correct result.

4. **RESPONSE TIME**
   Time necessary for having 95% of the response.
Electrochemical oligonucleotides biosensor

- Simple
- Fast
- Sensitive
- Specific
- Inexpensive
Electrochemical DNA biosensor

- **Steps involved in electrochemical DNA hybridization biosensors:**
  - Formation of the DNA recognition layer
  - Actual hybridization event
  - Transformation of the hybridization event into an electrical signal
Immobilization of DNA probe onto transducer surface

- simple adsorption onto carbon surfaces
- Immobilized of DNA in Carbon paste
- Use of biotylated DNA for complex formation with a surface-confined avidin or strepavidin
- Covalent linkage to the gold surface via functional alkanethiol-based monolayers
- Covalent (carbodiimide) coupling to functional groups on carbon electrodes
ADSORPTION
Covalent linkage

Biotinylated Molecule

avidin

Au surface
Covalent linkage carbodiimide
Covalent linkage carbodiimide

Substrate

Step 1: EtOH treatment, 5 min at RT
Step 2: 1% (wt/vol) KOH, 10 min at RT
Step 3: O₂⁻ plasma, 3 min

Step 4: 2% (vol/vol) APTES, 1 h at 80°C in vacuum desiccator

EDC, ssDNA
Covalent linkage Thiolated DNA
Effective factors on hybridization

- Ionic strength
- Temperature
- Length of sequence Covalent linkage to the gold surface via functional alkanethiol-based monolayers
- Time
- Peptide nucleic acid (PNA)
Diagnostic methods for hybridization event

- Label based detection of DNA hybridization
  - redox intercalators to recognize dsDNA
  - DNA-mediated electron transfer using mediators
  - Enzyme labels were used to amplify the signal and improve the sensitivity (Peroxidase, Glucose dehydrogenase)

- Direct detection of DNA hybridization
Label based detection of DNA hybridization

1. \[ + \]
   \[
   \begin{array}{cccc}
   T & C & G \\
   A & G & T & C \\
   \end{array}
   \]
   PROBE

2. \[
   \begin{array}{cccc}
   T & C & G & A \\
   A & G & T & C \\
   \end{array}
   \]
   HYBRID
   No hybrid
   No intercalator accumulation

3. Label (intercalator)

4. Probe signal

5. Hybrid signal
Label based detection of DNA hybridization

Diagram:

1. Indicator accumulation

2. Voltammetric measurement of indicator
Examples for commonly used indicators in DNA biosensors

**HYBRIDIZATION INDICATORS**

- Ruthenium bipyridine
- Methylene blue
- Cobalt phenanthroline
Label based detection of DNA hybridization
Label with enzyme based detection of DNA hybridization
Label with nano particles based detection of DNA hybridization

A. Hybridization with metal tagged signalling probe
   → Direct measurement of the metal tag signal

B. Hybridization with metal tagged signalling probe
   → Dissolution of metal tag
   → Measurement of dissolved metal tag signal
   → Direct measurement of silver tag signal
   → Measurement of dissolved silver tag

C. Hybridization with Au tagged signalling probe
   → Silver enhancement
   → Dissolution of silver tag
DIRECT DETECTION
DIRECT DETECTION
Electrode system

K1 Working Electrode (sense & drive shorted)
Reference (sense)

Counter (drive)
Signal Ground (DC Common)

Unused K2 Electrode (sense & drive shorted)

Conductive wire
Teflon or glass
Carbon paste

Carbon paste electrode

Conductive wire
Graphite lead

Pencil graphite electrode
EXAMPLE
Sensitive DNA impedance biosensor for detection of cancer, chronic lymphocytic leukemia, based on gold nanoparticles/gold modified electrode
ELECTROCHEMICAL DNA BIOSENSOR EXAMPLE 2

- Amperometric DNA sensor using the pyrroquinoline quinone glucose dehydrogenase-avidin conjugate

Kazunori Ikebukuro, Yumiko Kohiki, Koji Sode *
Biosensors and Bioelectronics 17 (2002) 1075--1080
MATERIAL AND METHODS

- Pyrroquinoline quinone-dependent glucose dehydrogenase ((PQQ)GDH) for DNA hybridization labeling
- Detection via biotin-avidin binding
- Target and probe DNA sequence:
  - Target DNA: 5’-bio-TCGGCATCAATACATCTC-3’
  - Probe DNA: 5’-bio-GATGAGTATTGATGCGGA-3’
  - Control DNA: 5’-bio-CTGATGAACATACATCT-3’
MATERIAL AND METHODS (CONT’S)

Fig. 1. Scheme of the preparation of the DNA-immobilized electrode and the detection of DNA hybridization.
CONCLUSIONS

- Simple
- Fast
- Sensitive
- Specific
- Inexpensive
DNA BIOSENSOR NOT LIMITED TO DNA DETECTION, BUT MORE...
Biosens. Bioelectr. 17,1075—1080

Wang, J. 2000. SURVEY AND SUMMARY From DNA biosensors to gene chips. 
Nucleic Acids Res. 28(16), 3011-3016


MOLECULAR BEACON BASED OPTICAL FIBER DNA BIOSENSORS

‘Ligate and light’. Schematics diagram of real-time monitoring of the nucleic acid ligation process by a MB.
Cy3 and Cy5: fluorescent dyes
Fluorescence Resonance Energy Transfer (FRET)

with target: loop-target hybrid
Cy3 and Cy5 separated - no FRET

Fluorescence intensity

Intensity ratio
PIEZOELECTRIC DNA BIOSENSORS

- quartz crystal microbalance (QCM) transducers

Diagram:
- Immobilized DNA probe
- Target DNA
- Form duplex—mass increase
- Decrease in crystal’s resonance frequency
Frequency–time response of a PNA/QCM to additions of the target (T) and mismatch (M) oligonucleotides. The hybridization event results in decreased frequency, reflecting the increased mass of the crystal.