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DNA-Biosensors – applications in the field of genomics

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Abstract

With the keyword *Genomics* most people associate DNA-array technology although alternative technologies such as biosensors are available. Considering the commercial success and broad distribution of state-of-the-art array technologies biosensors will not be able to compete with these systems, however, for certain applications they offer interesting and advantageous alternatives. In this paper a few selected examples of DNA biosensors are discussed with regard to the application used for or the underlying technology.

Biosensors

By definition, biosensors are self-contained integrated devices using a biological recognition element (the biochemical receptor) which is retained in direct spatial contact with the transduction element [1]. Systems with additional separation steps, additional hardware or reagent addition are not considered biosensors. On the contrary DNA-array technology (see for example the technology of the company Affimetrix) is a complex technique with several hardware components, with washing and separation steps etc. and is thus different from biosensor techniques. The underlying principles of detection, i.e. detecting a hybridization event, however, are quite similar.

A typical hybridization experiment is shown in Fig. 1. After the immobilization of the DNA-probes and the removal of unbound DNA the target hybridizes with the probe. Finally, after an additional washing step, the double stranded DNA can be detected using for example the streptavidin-biotin system, a dig-label or a radioactive marker. Using biosensors this set of individual steps can be simplified or shortened significantly, depending on the assay and the transducer used.

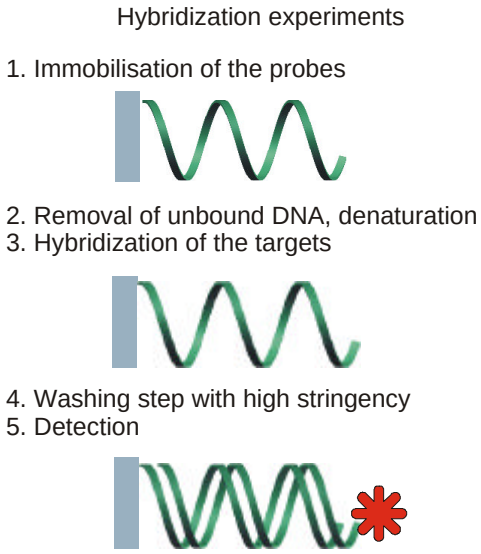

Fig. 1 Hybridization experiments – typical set of individual steps.

Depending on the application of the biosensor, the choice of a suitable transducer is extremely important. As a general rule, it can be concluded that almost every single transducer described in the literature has meanwhile been adopted to DNA-analysis. In Tab. 1 a few examples are given, for a more detailed review see for example [2, 3].

Tab. 1 Examples of DNA-biosensors and transducers used.

measuring principle	transducer	example
optical		
evanescent field	grating coupler	Bier, F. et al. (1997) *Sensors and Actuators* B, 38-39
reflectometric interference spectroscopy, RIfS	interference layer with spectrometer	Sauer, M. et al. (1999) *Anal. Chem.* 71
evanescent field, fluorescence detection	optical fiber with PMT	Kleinjung, F. et al. (1997) *Anal. Chim. Acta* 350
SPR	BIAcore	Nilsson P. et al. (1999) *BioTechniques* 26
evanescent field, fluorescence detection	PMMA prism with PMT	Peter et al. (2001) *Fres. J. Anal. Chem.*, submitted
electrochemical		
amperometry	carbon screen-printed electrodes	Marrazza G. et al. (1999) *Biosens. Bioelectron*. 14
amperometry	microelectrode array	Livache T. et al. (1998) *Biosens. Bioelectron*. 13
piezoelectric		
mass change	quartz cristal	Wang I. et al. (1998) *J. Am. Chem. Soc.* 120

Among the transduction principles most often optical and electrochemical transducer are used. An excellent review about electrochemical DNA-biosensors has been written by Palecek and Fojta [4]. Electrochemical transducers offer certain advantages such as their low cost, the simple design, small dimensions and low power requirements. In the following section, some examples of optical DNA-biosensors will be presented.

Using the BIAcore sensor – direct optical sensing

An interesting approach of a direct optical biosensor (the BIAcore) is described by Nilsson et al. [5]. The authors report about a new approach for mutational scanning of PCR products through hybridization analysis between complementary oligonucleotides. In the assay sets of overlapping probe oligonucleotides are hybridized to microbead immobilised PCR products. Mismatch-hybridisations between a mutant sample and the probes results in higher remaining concentrations in solution of the involved probe oligonucleotide. In a subsequent experiment with the BIAcore device the post-hybridization supernatants are analysed for the probe composition. This “subtractive oligonucleotide hybridization assay” allows for the construction of composition diagrams revealing the existence and approximate location of a mutation. As an example, the authors present data obtained with single mutations in exon 6 and 7 of the human p53 tumor-suppression gene.

Optical sensor for real-time hybridization analysis

Recently, at ICB in Münster an optical DNA-sensor was developed which is based on an evanescent-field excitation of fluorophors and which allows for real-time analysis of the hybridization event [6]. The system consists of 2 components, the disposable sensor chip and the optical read-out device. The assay format is shown in Fig. 2. In the sensor chip the oligonucleotide probes are immobilized on the surface of a glass plate. In the device, an evanescent-field is generated by total internal reflection of a laser beam. When the sample with the corresponding target is added, the evanescent field causes an excitation of fluorescence light only at those targets in very close proximity to the surface. As the fluorophors in the bulk solution are not excited washing or separation steps are not required. After the start of the measurement, the binding of the labeled targets to the immobilized probes can be observed in real-time. With a flow rate of 0.5 µl/s a whole measurement takes a few minutes only. Under the experimental conditions chosen, the initial binding is dependent on the concentration of the target only. Thus, not only the presence but also the concentration of a given target can be determined.

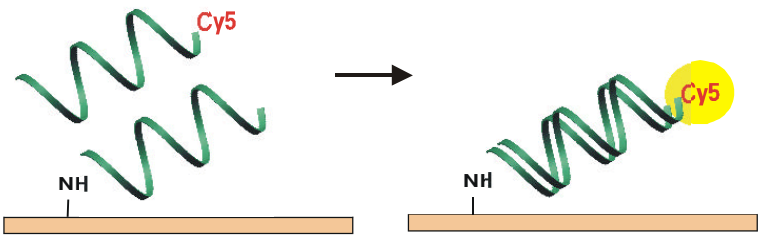

Fig. 2 Assay scheme for real-time hybridization analysis.

With a 5x5 array lower limits of detection for various oligonucleotide targets are approximately 5 pM. Using the right assay conditions (high stringency) even single-mismatches can clearly be distinguished from perfect matches by the initial binding kinetics. Currently, the system is adapted to gene expression analysis. Using in-vitro transcription for amplification the system will be trained to differentiate between tumor and normal tissue.

The combination of evanescent field excitation and fluorescence detection allows for sensitive and rapid measurements. At the same time, some of the disadvantages of direct optical sensors such as problems with unspecific binding or limited sensitivity are avoided.

Fiber-optical sensor array

Very interesting work on fiber optical sensors is done in the group of David Walt at Tufts University in Medford, Massachusetts. In work from Ferguson et al. [7], for example, a fiber-optical biosensor array is described for the simultaneous analysis of multiple DNA sequences. A bundle of optical fibers was assembled with each fiber carrying a different oligonucleotide probe immobilized on its distal end. Hybridization of complementary targets was then observed by an increase in fluorescence. A further development was described by Michael et al. [8]. In this paper an array based sensor together with a new approach for array fabrication is described. An application of this technology for DNA-analysis is presented by Ferguson et al. [9]. With this technology, sensor arrays are prepared by randomly distributing a mixture of microsphere sensors on an optical substrate containing thousands of micrometer-scale wells. As the sensors occupy a different location from array to array the identity of each sensor is ascertained and registered using encoding schemes rather than by a predetermined location in the array. The approach thereby shifts the demand from fabrication to signal processing. In the work of Ferguson et al. [9] the encoding is achieved by using internal and external encoding of the microspheres. For internal encoding europium dye in increasing concentrations was added to the beads. For external encoding, prior to the coupling of the oligonucleotide probes, two dyes (Cy5 and TAMRA) in various concentrations were coupled to the outer side of the microspheres. Whereas by entrapping the Eu-dye eight distinguishable microsphere families were prepared, the additional external encoding led to a library of 100 spectroscopically distinguishable microsphere types. A scheme of array assembly is shown in Fig. 3.

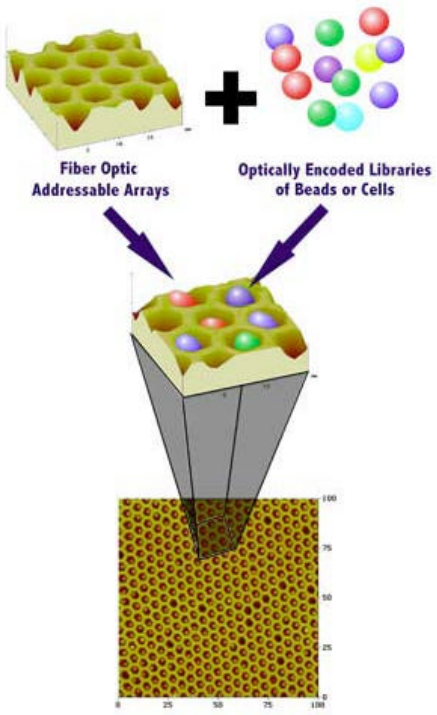

Fig. 3 *BeadArray™ technology of Illumina (www.iullumina.com). To form an array, each fiber optic bundle is typically dipped into a pool of coated beads. The coated beads are drawn into the wells, one bead per well, on the end of each fiber in the bundle. The ten thousands of beads at the end of the fiber optic bundle comprise the BeadArray™.*

To demonstrate the applicability for DNA analysis 25 microsphere families were modified with 25 different oligonucleotide probes. The encoding signals were then used to positionally register the microspheres in the array. After registration, the array was interrogated with samples containing the corresponding targets. One of the major advantages of this technology include cost-effective production of the microbead arrays in seconds, high-throughput analysis, easy replacement with additional or different microspheres when different testing is desired and facile regeneration to the sensor and the substrate.

Currently, this technology is commercialized by the American company Illumina (www. Illumina.com). In a joint development with Applera Corporation a next generation tool that will permit the large-scale analysis of genetic variation and function is developed.

Conclusions

Although only a few selected examples could be presented, looking at current DNA biosensor technology a few general conclusion can be made. First, it seems to be obvious that only a few DNA biosensors have the potential to compete with state-of-the-art DNA array technologies. Probably, the

applications of most DNA biosensors will be limited to certain niches. Possible applications range from the detection of genetically modified organisms to infectiology or genotyping. Although a clear trend towards systems with a higher density of probes (either conventional arrays, SPR imaging or fiber bundles) can be observed, only a few sensors described in the literature are beyond the proof-of-principle. For the future, however, several interesting technologies and applications thereof can be expected.

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