We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,300

Open access books available

172,000

International authors and editors

190M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

A Novel Type of Nucleic Acid-based Biosensors: the Use of PNA Probes, Associated with Surface Science and Electrochemical Detection Techniques

Eva Mateo-Martí¹ and Claire-Marie Pradier²

¹*Centro de Astrobiología (CSIC-INTA) Ctra. Torrejón-Ajalvir Km.4, 28850 -Torrejón de
Torrejón de Ardoz-Madrid,
Spain*

²*Laboratoire de Réactivité de Surface, UMR CNRS 7197, Université Pierre et Marie
Curie, 4, Pl Jussieu, 75005-Paris,
France*

1. Introduction

DNA biosensors are now recognized as invaluable tools for detecting target genes responsible for diseases, or pollution, in various fields (Fodor et al., 1991; DeRisi et al., 1997; Landegren et al., 1998). Therefore, detection of DNA hybridisation is of significant scientific and technological importance, as manifest, for example, from the growing interest in chip-based characterisation of gene expression pattern and detection of pathogens. DNA biosensors technologies are currently under intense investigation owing to their great promise for rapid and low-cost detection of specific DNA sequences. These technologies commonly rely on the immobilisation of a single-strand (ss) DNA probe onto optical, electrochemical, or mass-sensitive transducers (Xu et al., 1995; Mikkelsen et al., 1996; Okahata et al., 1992). There is still a crucial need to improve the sensitivity, selectivity and rapidity of the DNA hybridisation detection. Among the possible strategies, a new artificial Peptide Nucleic Acid (PNA), a highly specific probe, combined with the use of surface science techniques to optimise the sensing layer as well as to detect biomolecular recognition, open promising ways to win this biotechnological challenge. This chapter will focus on these two novel aspects of DNA biosensors.

1.1 PNA versus DNA comparison

Peptide Nucleic Acid was first described by Nielsen's group in 1991 (Nielsen et al., 1991). Knowledge of the PNA structure is essential for understanding its remarkable hybridisation behaviour and DNA recognition capacity (Figure 1).

PNA is an analog of DNA in which the entire negatively-charge sugar-phosphate backbone is replaced with a neutral « peptide like » backbone consisting on repeated N-(2-aminoethyl) glycine units linked by amide bonds (Figure 1). The four natural nucleobases (i.e., adenine, cytosine, guanine, and thymine) come off the backbone at equal spacing to the DNA bases.

Source: Intelligent and Biosensors, Book edited by: Vernon S. Somerset,
ISBN 978-953-7619-58-9, pp. 386, January 2010, INTECH, Croatia, downloaded from SCIYO.COM

Methylene carbonyl linkages connect the bases to the central amine of the backbone. Thus, PNA contains the same number of backbone bonds between bases and the same number of bonds between the backbone and the bases, as DNA. Such a structure offers a high biological stability, as it is not prone to degradation by nucleases or proteases (Demidov et al., 1994).

Owing to its neutral backbone and proper interbase spacing, PNA binds to its complementary nucleic acid sequence with higher affinity and specificity compared to traditional oligonucleotides. It was shown (Egholm et al., 1993) that PNA hybridisation to complementary oligonucleotides obeys the Watson-Crick base-pairing rules with the PNA and DNA strands joined via hydrogen bonds (see Figure 1). The neutral backbone also implies a lack of electrostatic repulsion between the PNA and DNA strands (compared to that existing between two negatively-charged DNA oligomers), and hence a higher thermal stability of PNA/DNA duplexes. It was also demonstrated that the thermal stability of the resulting PNA/DNA duplex is essentially independent of the salt concentration in the hybridisation solution (Orum et al., 1995; Wang, 1998).

(ss)PNA oligomers exhibit superior hybridisation properties, as well as improved chemical and enzymatic stability, compared to natural nucleic acids. For many applications, the synthetic DNA mimic, PNA, could be advantageously used as a probe molecule, owing to its unique physicochemical and biochemical properties. The unique structure and the resultant hybridisation properties of PNA, open up many important biological and diagnostic applications, not achievable with traditional oligonucleotides. In addition, the different molecular structures of DNA and PNA (see Figure 1), offer a large set of chemical signatures to be detected after hybridisation, thus allowing the use of label-free techniques of detection. The chapter will explain why and how these unique properties of PNA may be applied to DNA specific recognition.

Fig. 1. Schematic chemical model of PNA and DNA molecules, showing the different backbone linkages, (Mateo-Marti et al., 2007).

When searching to optimise biosensors, it is crucial to characterise all steps of their elaboration, as well as to detect, and possibly quantify, with the highest possible sensitivity

and specificity, the reaction of molecular recognition onto the receptor. Of course, we are talking about a characterisation at a molecular level, some properties being determining for the biosensor parameters: the surface coverage, the orientation/conformation of the adsorbed molecules, their spatial arrangement and, possibly, their charge. Since this chapter is focused on PNA-DNA systems, most of the time involving rather short oligonucleotides, surface science techniques are particularly well suited, though still rather seldom used in this domain.

1.2 Novel detection techniques for PNA-DNA-based biosensors

The unique properties of ssPNA and its physico-chemical differences with respect to DNA, encourage the use of surface characterisation techniques for the detection of label-free nucleic acid targets in biological samples. Under the term “surface” techniques, we will include electrochemical, gravimetric techniques, Quartz Crystal Microbalance (QCM), as well as specifically surface science characterisation techniques like X-ray Photoelectron Spectroscopy (XPS), Reflection Absorption Infrared Spectroscopy (RAIRS), Surface Plasmon Resonance (SPR), Surface Enhanced Raman Scattering (SERS) and Time-of-Flight Ion Mass Spectrometry (ToF SIMS); for the first time, these techniques, rather original when applied to PNA-DNA detection, will be gathered and compared in one chapter.

Electrochemical detection of DNA binding to DNA or PNA has been widely described in the literature (Liu et al., 2005; Steichen et al., 2007; Fang et al., 2008) Cyclic Voltametry (CV), or more sophisticated, Differential Pulse Voltametry (DPV), Electrochemical Impedance Spectroscopy (EIS), conductivity changes may be used as sensitive transduction techniques. The principles of these electrochemical techniques will be recalled; the advantage of using redox markers or electroactive labels will be considered. Often associated with impedance measurements, QCM, but also SPR, based on the detection of refraction index variations, have been successfully used to identify DNA hybridisation in real time.

Less commonly used for PNA-DNA hybridisation detection, though very sensitive, are the specifically surface science techniques, like XPS and RAIRS. XPS, though not applicable to instant, on line detection, has been applied to the characterisation of PNA, and then PNA + DNA layers, thus bringing valuable chemical information about the density/integrity of these molecular layers at these two steps (Mateo-Marti et al, 2005; Mateo-Marti et al, 2007). Surface infrared, RAIRS, whose sensitivity is enhanced on planar surfaces, is a new, very easy to handle, chemical detection technique.

Major drawbacks of current well-standardized molecular biology techniques are their time requirements for sample processing and amplification, risk of false positives from sample or reagent contamination, heavy instrumentation, laboratory costs and need for labeling of the analytes (Gau 2005). To overcome these drawbacks, a lot of research work is focussed on the development of label-free DNA biosensors, which allow the direct detection, and specific identification of point mutations in genes. Detection methods that avoid labeling of the target molecule to be hybridised usually guarantee a higher accuracy of the measurements (Buetow et al., 2001; Briones et al., 2005).

The PNA biosensor technology holds promises for rapid and cost-effective detection of specific DNA sequences. A single-strand nucleic acid probe is immobilised onto optical, electrochemical, or mass-sensitive transducers to detect the complementary (or mismatch) strand in a sample solution. The response from the hybridisation event is converted into a measurable signal by the transducer. We describe here the use of PNA as a novel probe for

sequence-specific biosensors and highlight some of the advantages of using it as a recognition probe.

This chapter presents a review of recent studies, focussed on PNA-DNA hybridisation detection by using “chemical”, or purely “surface science” techniques; the latter provide alternatives for optimizing nucleic acid-based sensors and expanding the range of detection methods available; the conditions of their use for this specific application as well as a comparison of their respective performances will be attempted and, moreover some indications helping to choose the best technique for the best detection, depending on the scientific case, will be suggested, based on the most recent literature survey.

2. Techniques and results

2.1 Surface spectroscopies

2.1.1 X-Ray Photoemission Spectroscopy (XPS)

XPS is one of the most universal techniques used for analysing surfaces from a chemical point of view. The bases of the technique lie in Einstein’s discovery of the photoelectric effect, whereby photons can induce electrons emission from a solid provided that the photon energy ($h\nu$) is greater than the work function (the work function of a solid is defined as the minimum energy required to remove an electron from the highest occupied energy level in the solid to the vacuum level). The vacuum level may be used as an “energy zero”. Surface analysis by XPS is accomplished by irradiating a sample with monoenergetic soft x-rays and analysing the energy of the detected electrons. These photons have a limited penetrating power in a solid of the order of 1-10 micrometers (Moulder, 1992) and, overall, the electron have a free path in solids of the order of 5-10 nm which makes XPS a true surface sensitive technique. They interact with atoms in the surface region, causing electrons to be emitted by the photoelectric effect. The emitted electrons have measured kinetic energies (KE) given by:

$$KE = h\nu - BE - Q_s$$

Where $h\nu$ is the energy of the photon, BE is the binding energy of the atomic orbital from which the electron originates, and Q_s is the spectrometer work function.

The binding energy may be regarded as the energy difference between the initial and final states after the photoelectron has left the atom. Hence it is clear that for a fixed photon energy, photoemission from an atom which well-defined core levels (of particular binding energy) will produce photoelectrons with well-defined kinetic energies varying systematically from one element to another (Attard & Barnes, 1998). Because each element has a unique set of binding energies, XPS can be used to identify and determine the concentration of the elements in the surface layers. Variations in the elemental binding energies (the chemical shift) arise from differences in the chemical potential and polarisability of compounds. These chemical shifts can be used to identify the chemical state of the materials being analysed. XPS is also a probe of the chemical environment or oxidation state of surfaces species. The detection limit for surface impurities in XPS can, in favourable circumstances, be less than 1% of a monolayer.

Structural-chemical differences between PNA and DNA molecule, encourage the use of XPS surface to detect and quantify the hybridisation.

In the following example, ssPNA molecules on gold surfaces, were adsorbed from water, forming a layer stable in the air, and showing efficient and specific ssDNA recognition capability. The quality of the sensing PNA layer, and then, the biological activity of the

SAMs of ssPNA and their interaction with complementary ssDNA have indeed been investigated by XPS. Immobilisation of ssPNA (1 μ M) on polycrystalline Au layers was performed at 22°C for 3.5 h in a humid chamber. After immobilisation, the gold samples were rinsed in H₂O at 22°C; at this optimal concentration, 1 μ M, PNA molecules realign their molecular axes with the surface normal and form SAMs without the need of co-immobilisation of spacers or other adjuvant molecules (Mateo-Marti et al., 2005). PNA hybridisation with 100 μ M ssDNA (2005). Hybridisation with 100 μ M ssDNA target solution was carried at 54°C for 1 h, in a buffer pH 7.2. Post-hybridisation washing was performed. Finally, blowing helium gas dried the gold samples. XPS was also performed to estimate the dynamic range of the recognition process of ssPNA by ssDNA. For PNA concentrations ranging from 0.1 to 1 μ M, an average increase of the normalized N1s peak ranging from 2.6 to 3.2 times after hybridisation was observed. The number of N atoms per ssPNA molecule is 64, versus 116 in the complementary DNA. An enhancement of the N signal by a factor of 2.8 may thus be expected from an approximate atom-counting model. A good agreement between the expected and obtained values suggests that, under optimal conditions, the fraction of ssPNA hybridised with DNA target is near 100%. This value is higher than that reported for DNA-DNA hybridisation measured with the same technique (Casero *et al.* 2003, D.Y. Petrovykh *et al.*, 2003). For PNA concentrations higher than approximately 5 μ M, the surface is completely covered by ssPNA and the photoemission signal does not change after hybridisation, indicating that the bioSAM is not behaving as an active biosensor. It can be concluded that bioSAMs of ssPNA offer optimal biosensor capacity when immobilised at concentrations of up to 1 μ M, thus forming non dense sensing layers.

The P signal, related to the phosphate groups in the DNA backbone but absent in PNA, together with an increase in the N signal detected after hybridisation, are the fingerprints of the hybridisation of complementary ssDNA to bioSAMs of ssPNA, in the XPS detection (Briones et al., 2004; Mateo-Marti et al., 2007). Moreover, the N(1s) core level peak was analysed at high resolution to search for a chemical shift due to the PNA-DNA hybridisation process. A spectral shift of 0.4 eV to higher binding energies, was indeed observed. The shift of the N (1s) peak to higher energy values has been ascribed to the hydrogen bonds, occurring in the hybridisation process, thus also constituting a fingerprint of the DNA to PNA binding.

Silicon, that represents a remarkable compromise between stability, biocompatibility, and maturity of the semiconductor processing technology, was also used for nucleic acid biosensors, combined with XPS characterisation. Cattani-Scholz et al. reported a stepwise functionalization of SiO₂-terminated Si surface by phosphonate and siloxane films modified with polyethylene glycol (PEG) and PNA as selective probes for DNA. Hybridisation has been characterised by XPS among others techniques; the biofunctional interfaces exhibit good resistance against non specific interactions, having potential applications for PNA/DNA microdevices. (Cattani-Scholz et al., 2008; Cattani-Scholz et al., 2009).

2.1.2 Reflection Absorption Infrared Spectroscopy (RAIRS)

Infrared spectroscopy may also be employed to identify the chemical nature and conformation of molecules adsorbed on surfaces. The fact that infrared spectroscopy provides specific information on the types of bonds present in a molecule, is non-

destructive, and does not require Ultra High Vacuum has made it a highly versatile technique for surface analysis. Moreover, the majority of vibrations of functional groups within adsorbed molecules generally occur above 800 cm^{-1} . To characterise adsorption and reaction phenomena occurring at the surface of a metal or of a semi-conductor, transmission experiments are not relevant; they can be advantageously replaced by applying IR spectroscopy in the reflection mode, where the sample is acting as a mirror, reflecting the beam (Attard & Barnes, 1998). RAIRS uses infrared light to excite the internal vibrations of the adsorbed molecules. The frequency of these vibrations can reveal the chemical groups in the adsorbate, the adsorption geometry and the adsorption site occupied by the surface molecule.

Classically, the general infrared selection rule states that *for a vibration to be infrared active, the electric dipole moment of the molecule must change during the vibration*. While for a molecule adsorbed on a metal surface, the interaction of the infrared radiation with the adsorbate dipole is influenced by the dielectric behaviour of the metal. This gives rise to the following selection rule for RAIRS:

‘ONLY VIBRATIONAL MODES WITH A DIPOLE MOMENT CHANGE NORMAL TO THE SURFACE WILL BE OBSERVED’

An infrared spectroscopy experiment consists in measuring the differences between the light intensity of the incident and transmitted beams of radiation in a particular frequency region; for RAIRS the difference between light reflected on a clean and modified surfaces will be measured. Because of the extremely highly inherent resolution of RAIRS (less than 4 cm^{-1}) inspection of the spectra for simple molecules yields unique information about the chemical state and interactions, H-bonding or charge transfer for example.

The different molecular structures of DNA and PNA offer a wide range of chemical signatures to be detected only after hybridisation, thus allowing the use of RAIRS as detection technique. Figure 2 shows, a comparison between PNA and PNA-DNA infrared spectra on gold surfaces, some features are enhanced, and also, the characteristic bands of DNA molecular groups, appear. These findings indicate the possibility to detect a hybridisation process by IR (Mateo-Marti et al., 2007).

The various chemical functional groups present in each molecule, PNA and DNA have been identified and associated to specific infrared features. Vibrational modes associated with nucleobases (heterocyclic) and vibrational features associated with the backbone (CH_2 alkane group, -O-ether functionality, NH-CO amide group). Regarding the nucleic bases, the main features should appear in the following regions (Mantsch, 1996): the in-plane (ip) double bond vibrations of the bases are located at $1780\text{--}1500\text{ cm}^{-1}$, base-deformation motions appear at $1500\text{--}1250\text{ cm}^{-1}$, and out-of-plane (oop) base vibrations at frequencies lower than 1000 cm^{-1} (see Figure 2).

Two features are specific fingerprints of the DNA, as no phosphate groups are present in the PNA molecule. The appearance of two strong absorption bands, assigned to the phosphate vibrations (antisymmetric PO_2^- stretching vibration at 1237 cm^{-1} and symmetric stretching vibrations at 1081 cm^{-1}) can be clearly identified on the spectrum (see Figure 2). They are reliable proofs of the PNA-DNA hybridisation process; phosphates, and their respective infrared signals, should only be present when a nucleic acid has bound to the complementary PNA molecule.

Fig. 2. A comparison PM-RAIRS spectra of PNA and PNA-DNA after hybridisation process (Mateo-Marti et al., 2007).

2.1.3 Surface Enhanced Raman Scattering (SERS)

Surface Enhanced Raman Scattering (SERS) is a particularly sensitive surface analytical technique, taking advantage of the increased local field on nanostructured surfaces; the ideal substrates are typically Au or Ag. Researches using SERS on nanostructured surfaces and/or nanoparticles have given rise to spectacular results, in term of sensitive detection, applied for instance to biosensors; examples of DNA hybridisation are not common due to the difficulty to immobilise these probes on nanostructures in a controlled way.

Detection of DNA by SERS may also take advantage of the electrostatic interactions generated when the oligonucleotides interacts with PNA; this enables the binding of positively charged nanoparticles bearing a SERS reporter molecule for instance. In Fabris et al. study (Fabris et al., 2007), DNA was hybridised to PNA immobilised on slides, leaving a negatively-charged surface; immersion in a solution of positively-charged Ag Nanoparticles (NPs), and then in a solution of rhodamine SERS reporters, leads to intense SERS signal, attributed to the specific PNA-DNA interaction. Note that addition of non-complementary ssDNA, at the initial step, leaves the PNA layer unmodified, thus not binding nanoparticles; a resulting none or quasi no SERS signal has been observed (see Fig. 3).

Another way to detect DNA hybridisation by SERS was recently described by Fang et al. It consisted in using ordered arrays of silicium nanostructures, coated with gold or silver as SERS substrates; the latter were fonctionnalised with PNA and submitted to target DNA. Thanks to the affinity of the DNA phosphate groups towards Zr⁴⁺ cations, these oligonucleotides, immersed in zirconide chloride, were bearing Zr cations and this only on PNA-duplex; after incubation in Rhodamine B that reacts with Zr cations, the hybridisation

Fig. 3. General scheme of the assay, PNA in green and complementary DNA in blue. SERS signals from PNA slides hybridised with ssDNAc (blue) or ssDNAnc (red) (Fabris et al., 2007).

could be detected by SERS (Fang et al., 2008a), with a detection limit of 1×10^{-12} M. Of course, the sensitivity increases with the oligonucleotide length due to the higher amount of phosphate groups. Note that all Raman measurements were obtained in the air, after sample rinsing; this appears not to perturbate the data. The selectivity of this assay was checked by testing a single base mismatched DNA; no signals were observed in that case.

This method, enabling sensitive SERS detection, is applicable to other biological recognition systems, but our purpose is to show here the interest of using neutral PNA as the specific probe for DNA; commercial PNA-slides now even exist (Ujihara et al., 2005).

2.1.4 Time-of-Flight Ion Mass Spectrometry (ToF SIMS)

Worth mentioning are some recent studies, using ToF SIMS, an ultra sensitive surface analysis technique to detect unlabeled DNA targets on PNA microarrays (Arlinghaus & Kwoka, 1997; Brandt et al., 2003). The experiment consists in bombarding the sample with an ion beam that induces molecular fragments and/or ions from the topmost surface layer to desorb. They are then detected by a ToF mass spectrometer. In Arlinghaus et al. work, ToF-SIMS was used to monitor the amount of immobilised PNA on platinum-coated silicon chips, and thus check the sample reproducibility. These chips were then hybridised and analysed for phosphorous signal; a peak was observed in the case of complementary

nucleotides and no peaks at the non complementary spot, demonstrating the successful selective hybridisation. The method takes advantage of its great sensitivity to PO_2^- and PO_3^- fragments of the phosphate ions present on DNA (and not on PNA).

2.2 Optical and gravimetric techniques

2.2.1 Surface Plasmon Resonance (SPR)

Surface Plasmon Resonance (SPR) is a surface analysis technique that measures changes in the refractive index that occur upon adsorption on a gold film.

Fig. 4. Principle of Surface Plasmon Resonance Spectroscopy, a label-free detection technique (Brandt & Hoheisel, 2004).

The first commercial instrument was launched in 1990 by Biacore to assay molecular recognition on a planar surface; since that date the sensitivity and handling have been improved and adapted to specific cases making the technique very powerful in a wide range of applications, food or environmental monitoring, detection in medicine for example (Homola, 2003; Karlsson, 2004).

DNA hybridisation to PNA probes, immobilised on a planar gold surface, may be measured by using Surface Plasmon Resonance (SPR). In the latter case, DNA binding to PNA induces a change in the refractive index, proportional to the mass uptake on the chip surface and enabling a real-time monitoring of the hybridisation process (Jensen et al., 1997). A small review of some PNA-based biosensors has been recently published, presenting some examples of biosensors using labels or not, and showing the interest of using these DNA mimics (Brandt & Hoheisel, 2004; Corradini et al., 2004); they report in particular that PNA monomers may be modified by adding a chiral center to enhance their mismatch recognition sensitivity by SPR (Corradini et al., 2004); also, instead of using the direct grafted PNA format, PCR products may be attached to the SPR sensor surface, followed by PNA oligomer hybridisation leading to highly efficient PNA hybridisation; this has been attributed to the better accessibility of PNA probes to the immobilised PCR, compared to that of DNA oligonucleotides (Feriotto et al., 2001).

However, the SPR detection sensitivity often needs to be enhanced by incorporating labels or using a second hybridisation (sandwich format). Using Au Nanoparticles labels, Yao et al. report a very high sensitivity of the SPR technique in DNA hybridisation, down to the femtomolar level (Yao et al., 2006). Another example is reported by Su et al. who describe a method relying on the use of charge neutral peptide nucleic acids as capture probes and polymer-modified DNA as targets: protonated aniline monomers, that do not adsorb on neutral PNA (Fan et al., 2007a), are first adsorbed to the DNA molecules; polymerisation

into polyalaline then occurs and, enhanced refractive index changes are observed when this DNA-polyalaline hybridises to PNA (Su et al., 2008); the lowest concentration of DNA, detectable in this way by SPR, was 0.1 pM which is close to that attained by fluorescent methods (Will et al., 1999).

More recently, a Localised Surface Plasmon Resonance (LSPR)-based label-free biosensor utilising gold-capped nanoparticles, modified with DNA probes, was developed. The optical properties of gold nanoparticles, measured from the absorbance strength, are modified upon DNA hybridisation. The LSPR-based biosensor was made of a gold-coated glass substrate on which 100 nm silica nanoparticles were deposited and then capped with gold leading to a full and homogeneous layer of nanoparticles; the SPR absorbance, at ca 537 nm, increased upon increasing the adsorbed molecular layer, e.g. upon DNA binding to PNA probes; this system permitted to reach a limit of detection equal to 0.7 pM target DNA, with a good sequence selectivity (Endo et al., 2005).

Interestingly, the kinetics and affinity constants of the DNA-DNA and PNA-DNA pairings were compared, using chromophore-labeled oligonucleotides and SPR detection; the two systems, where the probes were surface-attached via biotin-avidin linkages, showed similar affinity constants, 2 to $3 \times 10^7 \text{ M}^{-1}$, but PNA-DNA binding showed some deviation from the classical Langmuir DNA-DNA model; this was interpreted by a first binding of DNA to uncharged PNA followed by a rearrangement on the surface; this simply underlines, that by using an uncharged oligonucleotide, important changes in the structure/conformation of the duplex may occur and change the kinetics of the hybridisation reaction (Kambhampati et al., 2001).

Surface Plasmon Fluorescence Spectroscopy (SPFS) is another derivative of SPR, which we will not detail in this chapter; let's just mention that this technique was applied to detect PNA-DNA hybridisation and characterise the kinetic parameters of this system. The authors confirm possible structural changes of PNA-DNA duplexes after hybridisation, strongly influenced by the ionic strength of the solution (Liu et al., 2006).

2.2.2 Quartz Crystal Microbalance (QCM)

Quartz Crystal Microbalance (QCM) technique measures, in real time, changes in mass of the surface of a resonating quartz, by monitoring its resonance frequency; it gives information similar to that obtained by SPR, but also, it provides precious data about the kinetics of interaction and about the structure of the adsorbed layer by measuring dissipation energy. Obtaining these complementary sets of information is unique and permits valuable discussions about correlating the reactivity/sensitivity of a sensor to the immobilisation method for example (Höök et al., 1998).

F. Höök et al. developed a QCM-based PNA-DNA sensor, showing the interest of measuring the frequency and dissipation changes simultaneously for studying various ways of immobilising PNA probes (Höök et al., 2001). The authors immobilised PNA probes in two different ways, either directly to the gold quartz via a thiol function, or on an intermediate biotin-avidin layer. Interestingly, responses observed upon DNA hybridisation were different: thiol-PNA forms a rigid structure with essentially no affinity toward complementary DNA, while biotin-PNA, bound to a 2D arrangement of streptavidin, forms a flexible layer with a high affinity to complementary DNA. Note that a single mismatched DNA was also recognised, but to a lesser extent and overall, its binding was reversible conversely to that of the fully complementary oligonucleotide. Eventually, the authors

compared the PNA-DNA and DNA-DNA layers, by measuring the changes in frequency and dissipation when binding DNA; it appears that biotin-DNA forms a more expanded layer than biotin-PNA, with very little dissipation change upon DNA hybridisation (see the $\Delta D = f(\Delta f)$ curve, fig. 5); biotin-PNA forms a flexible but less expanded layer, with higher dissipation change upon DNA hybridisation; this difference is obviously related to the interactions between charged or uncharged oligonucleotides.

Fig. 5. ΔD versus Δf curves for biotin-DNA and biotin-DNA binding to streptavidin, followed by DNA hybridisation, from (Höök et al., 2001).

The high sensitivity of Quartz Crystal Microbalance was demonstrated by Wang et al. who measured the hybridisation of single-base altered DNA strands, even in the presence of a large excess of single-base mismatch oligonucleotides (Wang et al., 1997a). They estimated the detection limit, in the case of a 15 mer-long probe, around 1 $\mu\text{g/ml}$.

In a more recent study, QCM measurements were combined to SPR analyses in order to circumvent the problem raised by changes in the refractive index when denaturants, or potential changes, are applied to facilitate single mismatch detection (Lao et al., 2009). When 22-mer probes are involved, hybridisation to both PNA and DNA probes occurs but PNA probes show a higher efficiency for single mismatch discrimination. The authors demonstrate that PNA probes are more stable and efficient in mismatch identification, even when dehybridisation is assisted by potential changes.

Another type of chemistry was applied to immobilise single-stranded DNA molecules at the air-water interface, using cationic Langmuir monolayers (Ramakrishnan et al., 2002; Sastry et al., 2000). Interestingly, the hybridisation of complementary PNA molecules was achieved at this interface, leading to very stable DNA-PNA hybrid monolayers. The PNA-DNA hybridisation was measured by QCM; again, in the case of a single mismatch DNA sequence, no hybridisation with PNA was observed. Note that the stability of these hybrid complexes was investigated by measuring the absorbance as a function of temperature, showing an enhanced stability on the cationic layer; this has been ascribed to the screening of the DNA charges by the monolayer, thus stabilising the duplex. This approach demonstrated first that DNA or PNA may be immobilised and hybridised successfully at an air-water interface and second, the importance of the attachment layer; in the presented

case, the cationic monolayers screened the electrostatic interactions and leaves the bases of the DNA molecules well oriented and accessible thus favouring hydrogen bonding of the complementary PNA; this had never been observed in solution.

PNA-DNA interactions have been used to direct the assembly of nanoparticles into macroscopic arrangements. The quantification of the hybridisation efficiency was ensured by fluorescence (Chakrabarti & Klibanov, 2003).

In a very recent study, hybridisation of hepatitis B virus by PNA was detected by QCM with a detection limit of 8.6 pg/L (Yao et al., 2008). This work relied on the immobilisation of PNA probes on a gold quartz using Carruso et al. method, grafting of a thiol on which avidin was chemically bound and then the biotinylated PNA probe (Carruso et al., 1997). Hybridisation with complementary and mismatch sequences was then measured by monitoring the quartz frequency, showing a remarkable mismatch discrimination. The authors underline the paramount importance of the probe immobilisation procedure to ensure low non-specific interaction and high reproducibility. Eventually, the detection sensitivity was improved by coating complementary ssDNA probes with RecA protein, before hybridisation to PNA in the QCM cell.

2.3 Electrochemistry

Electrochemical concepts have also proven to be very useful for sequence-specific biosensing of DNA, because they provide simple, rapid, label-free and low-cost detection of nucleic acid sequences [Wang et al., 2000; F. Patolsky et al., 1999]. The binding of the surface-confined probe and its complementary target strand is translated into a useful electrical signal. Among the electrochemical methods, the most used is based on redox labels, which generate a signal change upon hybridisation. Of course, the main drawback of these techniques is the need for this redox label to be added in solution, or grafted on DNA strands. To solve this problem, the redox indicator can be covalently grafted onto the electrode. Following this scheme, electrochemical methods like Cyclic Voltammetry (CV), Differential Pulse Voltammetry (DPV) or Electrochemical Impedance Spectroscopy (EIS) have been successfully used (Reisberg et al., 2008 and ref. in).

Among the electrochemical sensors, we will report on those, which involve PNA immobilised on the electrode surface as a probe for the recognition of the target DNA. The electrical neutrality of the PNA backbone, the adsorption of PNA onto charged carbon or mercury electrodes differs greatly from that of DNA in terms of potential dependence, and surface packing. Therefore, the strong adsorption of PNA, combined with the electroactivity of the nucleobases, can be exploited for developing highly sensitive and selective sensors for measuring trace levels of DNA (Wang et al., 1996).

2.3.1 Potentiometry

The signal measured is the potential difference (voltage) between the working and the reference electrodes. The working electrode's potential depends on the concentration of the analyte in the gas or solution phase. The reference electrode is needed to provide a defined reference potential.

Direct methods rely on the intrinsic electrochemical properties of DNA (the oxidation of purine bases, particularly guanine), or in changes in some of the interfacial properties after hybridisation. The first report in which guanine oxidation chemistry was used for target detection was described by Wang et al., who showed how the chronopotentiometric signal

of guanine decreased after incubation of an oligo d[G] modified electrode with a guanine-free target (Wang et al., 1997b). Wang et al. have reported the use of PNA as a recognition probe for the electrochemical detection of the hybridisation event using chronopotentiometric measurements. The method consists of four steps: probe (PNA) immobilisation onto the transducer surface, hybridisation, indicator binding (Co(phen)_3^{3+}), and chronopotentiometric transduction (Wang et al., 1996).

A carbon-pasted electrode is in this process bearing the immobilised DNA or PNA probe. The hybridisation experiment was carried out by immersing the electrode into the stirred buffer solution containing a desired target, followed by measurement of signal. The detection limit was of 10 pmol of the 15-mer oligonucleotide target was observed following a 10 min hybridisation. PNA carbon-paste biosensor was used for the detection of specific mutation in the p53 gene, a mutation related to various types of cancer. Challenging, the PNA biosensor with single-base mismatch oligomer resulted in a 3% error, as compared to a 91% error for the DNA recognition (Wang et al. 1997c).

2.3.2 Voltametry

Voltametry is an electrochemical technique in which a controlled potential is applied to an electrode (the working electrode), and any current that results from an electron transfer reaction at the electrode is measured. A potentiostat is used to apply the desired potential to the working electrode, relative to a reference electrode, and to measure the resulting current. While it is possible to perform voltametry with just two electrodes, in practice a third electrode, the auxiliary electrode, is employed in the current measurement system. The potentiostat is designed to prevent any current flow at the reference electrode, which might result in changes to its electrochemical potential (Van Benschoten, 1983). There are different modes of voltametry depending of the information desired. Cyclic Voltametry (CV) is a form of linear sweep voltametry that is carried out at a stationary electrode in a quiet solution, and is an important technique for characterisation of electroactive species. In cyclic voltametry, a solution component is electrolysed (oxidised or reduced) by placing the solution in contact with an electrode surface, and then making that surface sufficiently positive or negative in voltage to force electron transfer.

Voltametry technique has been applied for PNA-DNA detection in several studies. PNA molecules are immobilised as capture probes on a gold substrate. The redox complexes do not interact electrostatically with the PNA probes due to the absence of the anionic phosphate groups on the PNA probes. But after hybridisation, the complex is adsorbed on the DNA backbone, giving a clear hybridisation detection signal in ac voltametry. The process combines the selectivity of the peptide nucleic acid recognition layer with the sensitivity of ac voltametry.

We describe several examples using complexes as redox markers, and different modes of applying voltametry as a detection technique. Aoki et al. have described the use of mixed monolayers of PNA and 6-mercapto-1-hexanol on gold disk electrodes to detect complementary oligonucleotides at micromolar level by cyclic voltametry. The system relies on the electrostatic repulsion of the diffusing ferrocyanide redox marker, accrued from the hybridisation of the negatively-charge target DNA and the neutral PNA probe. Binding of the complementary oligonucleotide to the PNA probe monolayer increases the negative charge at the surface of the electrode, which results in electrostatic repulsion between the redox marker $[\text{Fe(CN)}_6]^{4-/3-}$ in solution and the monolayer, thereby hindering the redox

reaction of the maker; this allows the indirect detection of the complementary oligonucleotide (Aoki et al., 2000).

Steichen et al. have reported that the cationic ruthenium complexes ($[\text{Ru}(\text{NH}_3)_6]^{3+}$), is adsorbed on the DNA backbone only after hybridisation, giving a clear hybridisation detection signal in ac voltametry (Steichen et al., 2007). Furthermore, new method for the PNA-DNA detection has been developed. Ferrocene-containing cationic polythiophene does not bind electroscally to the PNA probes due to the absence of the anionic phosphate groups before hybridisation. After the PNA-DNA hybrid is formed, ferrocene-containing cationic polythiophene interacts electrostatically with the negatively charged phosphate groups and is adsorbed on the DNA backbone, giving a clear hybridisation detection signal in the cyclic voltamograms (qualitative information) and Differential Pulse Voltametry (DPV) (quantitative information). DPV has a much higher current sensitivity and better resolution than cyclic voltametry, because DPV provides a convenient peak shape for quantification, and the compounds, that get oxidized at different potential, will show up as separate peaks if their oxidation potentials are sufficiently different (Adams et Justice, 1997; Fang et al., 2008b). Kerman et al. report on electrochemical biosensor based on PNA for detection of PNA-DNA hybridisation the oxidation signal of guanine at carbon paste electrode detected by using DPV (Kerman et al., 2003).

Square Wave Voltametry (SWV) is a very sensitive and fast technique (voltamograms can be obtained in a few seconds), therefore, very well suited to study DNA hybridisation (Liu et al., 2006). SWV have been used to detect and quantify PNA-DNA hybridisation, based on ferrocene (Fc)-streptavidin conjugates. The quantification of target DNA can be easily obtained on this sensor platform with good stability and reproducibility, and the PNA-DNA affinity constants can be obtained from the redox-labeled streptavidin coupled to the duplex. Another example of detection of the electrochemical changes using SWV, PNA were attached covalently onto a quinone-based electroactive polymer, following changes in flexibility of the PNA probe strand upon hybridisation generates electrochemical changes at the polymer-solution interface. An increase in the peak current of quinone was observed upon hybridisation of probes by the target DNAs, whereas no change is observed with non-complementary sequence. Hybridisation was detected by recording the modification of the redox process of the quinone group, using SWV (Reisberg et al, 2008).

Hashimoto et al. have described the elaboration of electrochemical PNA arrays for the detection of the cancer gene ras; PNA was immobilised on gold electrodes of the array, and by using Linear Sweep Voltammetry (LSV) can provide a quick and convenient method for determining target DNAs specifically and quantitatively, with a detection limit of 10^{-13} M (3 fmol) (Hashimoto, K. & Ishimori, Y. 2001).

The PNA-SAM sensor was tested to differentiate the A2143G point mutation of *H. pylori* versus the wild-type sequence oligonucleotide, showing a good selectivity and sensitivity. *H. pylori* is recognised as one of the most common pathogens afflicting humans, the presence of the bacterium in the gastric mucosa is associated with chronic active gastritis and is implicated in more severe gastric diseases (Steichen et al., 2007). The detection limit of the complementary DNA oligonucleotide is 80 pM. Currently a hybridisation volume of 20 μl of DNA target is exposed to the PNA-SAM electrode, meaning that 1.6 fmol of the complementary target can be detected. However the use of microelectrodes could significantly improve the detection limit. DPVs detection limit for PNA-DNA hybridisation is 1.0×10^{-8} M on a planar electrode and reproducible results Residual Standard Deviation

(RSD) of 5% was obtained for four repetitive measurements of hybridisation of ss-PNA probe with 4.0×10^{-8} M target DNA (Fang et al., 2008).

Fig. 6. (A) Schematic representation of the electrostatic DNA hybridisation detection method using $[\text{Ru}(\text{NH}_3)_6]^{3+}$ as redox markers, and PNA as capture probes on gold. (B) Imaginary admittances of the PNA sensor before (a) and after (b) hybridisation with $1 \mu\text{M}$ complementary DNA (from Steichen et al., 2007).

2.3.3 Electrochemical Impedance Spectroscopy (EIS)

Electrochemical Impedance Spectroscopy (EIS) is an experimental method to characterise electrochemical systems. This technique measures the impedance of a system over a range of frequencies; the frequency response of the system, including the energy storage and dissipation properties, is revealed. Often, EIS reveals information about the reaction mechanism of an electrochemical process: different reaction steps will dominate at certain frequencies, and the frequency response shown by EIS can help identifying the rate limiting step. Among the used electrochemical techniques, electrochemical impedance spectroscopy has been shown very effective and sensitive for the characterisation of biomaterial-functionalised electrodes and biocatalytic transformation at electrode surfaces.

The principle of impedimetric sensing of DNA hybridisation is based on the formation of nucleic acid/DNA complexes at an electrode surface. The resulting negatively charged

interface electrostatically repels the negatively charged redox indicator $\text{Fe}(\text{CN})_6^{3-/4-}$. The electrostatic repulsion of the redox-active molecules leads to an enhanced charge transfer resistance, R_{ct} . Thus, R_{ct} will increase with the increasing amount of hybridised DNA. Electrochemical impedance spectroscopy was already successfully used for the visualisation of DNA hybridisation. In EIS, the binding of the target strands to surface-immobilised capture probes is indicated by a shift in the impedance spectrum of the modified electrode [Katz et al., 2003]. In addition, label-free detection of DNA can be obtained by interaction of DNA with electrochemically active ligands [Wang 2003]. Liu et al. have studied in situ hybridisation kinetic studies with label-free DNA target oligonucleotides on a mixed monolayer of PNA and mercaptohexanol on Au electrodes using EIS. EIS can be used to follow the in-situ hybridisation kinetics of PNA/DNA by recording the change of R_{ct} with time (Liu et al., 2005). The immobilised PNA probes on the sensor surface are uncharged, and hence, do not affect the charge transfer from the redox indicator $\text{Fe}(\text{CN})_6^{3-/4-}$ to the electrode. Once DNA targets hybridise to PNA, the charge density at the sensor surface will be changed, being a probe to monitor the PNA/DNA hybridisation process. This experiment demonstrated for the first time that EIS is a simple and convenient technique for the in-situ kinetic analysis of DNA hybridisation. A single-base mismatch can be directly seen in the kinetic curves. Moreover, the exact association (hybridisation) and dissociation rate constants can be obtained. Thus, the method is comparable to a fluorescence technique (Liu et al., 2005).

Monolayers of cysteine-linked peptide nucleic acid assembled on gold electrodes, were investigated for the sensing of DNA recognition (Degefa et al., 2008). The monolayer was characterised by cyclic voltametry. The electron transfer through the monolayers was investigated using electrochemical impedance spectroscopy in the presence of target DNA and redox marker ions $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as hybridisation indicator. The sensing of DNA at the PNA SAMs was also investigated at different concentration of the target DNA. Degefa et al. show that at lower target DNA concentration, a lower charge-transfer resistance, R_{ct} , was observed, and as the concentration was increased the resulting charge-transfer resistance, R_{ct} , increased too. The PNA surface was also studied for the detection of mismatched target DNA, the fully matched DNA resulted in a higher charge transfer resistance, R_{ct} , than that of mismatched DNA target at higher target concentration. The non-complementary nucleic acid showed a much reduced effect at all target concentrations.

Keighley et al. present a detailed EIS-based study of the simultaneous co-immobilisation of thiol-modified PNA probes and mercaptohexanol (MCH) onto gold electrodes, an optimal density exists, corresponding to a maximum in the change of R_{ct} for the negatively charged ferri/ferrocyanide redox couple, upon hybridisation with target DNA (Keighley et al., 2008). The optimisation of PNA surface density and the measurement ionic strength are key factors to the success of electrochemical impedance spectroscopy for label-free detection of DNA hybridisation. A detection limit of 25 fmol target is demonstrated. This is likely to be further improved by reduction of the electrode area and sample volume, this would allow reducing the detection limit to 3 amol.

DNA biosensors, especially those based upon detection of the intrinsic negative charge of target DNA, can be greatly improved by the use of uncharged peptide nucleic acid probes. Hybridisation causes an increased electrostatic barrier for the negatively charged ferri/ferrocyanide redox couple, resulting in an increase in charge transfer resistance R_{ct} that is measured using electrochemical impedance spectroscopy.

3. Conclusion

PNA, an ingenious DNA mimic, has two main advantages compared to DNA as a probe: first, it has no phosphate ions making the latter unique labels for the presence of DNA; second, its backbone is uncharged permitting a better hybridisation and the use of other matrix molecule or polymer to increase the binding and detection qualities. PNA offers a promising opportunity for highly specific nucleic acid recognition.

In this chapter, we present a quick overview of some surface analysis techniques that have been successfully applied to the detection of PNA-DNA hybridisation. While some surface sensitive techniques are compatible with on line measurements, like all electrochemical techniques, SPR and QCM, some are applied after hybridisation reaction; this is the case of RAIRS, and of course XPS and ToF-SIMS that need vacuum conditions. It is clear from this overview that, whatever the selected detection technique, the way of immobilising the PNA probe is crucial; some gain in sensitivity were observed when the density and accessibility of PNA were controlled. One also saw that playing with the hybridisation of neutral PNA with positively charged DNA offers advantages, like binding nanoparticles, redox indicators or polymerisation of oligomers, that increase the detection sensitivity. Chemical differences in PNA and DNA backbones also provide with marked hybridisation “labels” that have been exploited by the XPS or RAIRS techniques.

Table 1 summarise the techniques, their principle and associated sensitivity from the literature overview. One main observation is that the reported sensitivity values are very much fluctuating and this because researches in the PNA-DNA field are still recent and, for some of them, focussing on the feasibility and technical developments for these rather novel

Technique	Basic Principle	Sensitivity	Reference
SPR	Change in the refractive index upon hybridisation	0.1 pM	(Su et al., 2008)
		0.7 pM	(Endo et al., 2005)
		A few nM	(Liu et al., 2006)
QCM	Change in the resonance frequency of a quartz	8.6pg/L	(Yao et al., 2008)
		5 µM	(Ananthanawat et al., 2009)
		10 µg/mL	(Wang et al., 1997)
		1 µM	(Höök et al., 2001)
SERS	Enhanced electromagnetic field on nanostructured surfaces	1 pM	(Fang et al., 2008)
ToF SIMS	Detection of PO ₂ ⁻ and PO ₃ ⁻ fragments		(Arlinghaus & Kwoka, 1997; Brandt et al., 2003)
RAIRS	PO ₂ ⁻ vibrational frequency	100µM	(Mateo-Marti et al., 2007)
XPS	Phosphate binding energy	100µM	(Mateo-Marti et al., 2007)
EIS	Impedance/frequency response	1 nM	(Keighley et al., 2008)
Potentiometry	Potencial difference (voltage)	10 pM	(Wang et al., 1997c)
Voltametry	current	3 fM	(Hashimoto & Ishimori, 2001)

Table 1. Summary of the techniques, their principle and their associated sensitivity for PNA-DNA hybridisation detection.

techniques in the domain. To our view, more important than sensitivity that very much depends on the measurement conditions and, in some cases, types of labelling, the use of synthetic PNA, instead of DNA, offers a remarkable specificity to a single mismatched oligonucleotides ; this is critical for many gene disease recognition. Let's keep in mind that using this remarkably specific PNA-DNA hybridisation process, is attractive for genetic diagnosis. Work is left to be done to improve the sensitivity, not forgetting that non specific events have to be kept silent.

4. References

- Adams, N.R. ; Justice, J.B. Jr. (1997). *Voltammetry in the neuroscience : Principles, methods and applications*. USA.
- Ananthanawat, C., Vilaivan, T., and Hoven, V. P. (2009). Synthesis and immobilization of thiolated pyrrolidinyI peptid nucleic acids on gold-coated piezoelectric quartz crystals for the detection of DNA hybridization. *Sens. Actuat. B* 137, 215-221.
- Aoki, H.; Buhlmann, P. and Umezawa, Y. (2000). Electrochemical detection of a one-base mismatch in an oligonucleotide using ion-channel sensors with self-assembled PNA monolayers. *Electroanalysis* 12, 1272-1276.
- Arlinghaus, H. F. and e. al. (1997). Analysis of biosensor chips for identification of nucleic acids. *Anal. Chem.* 17, 76-84.
- Attard, G. and Barnes, C. (1998) *Surfaces*, Oxford university press, New York.
- Brandt, O. and et. al; (2003). PNA microarrays for hybridization of unlabelled DNA samples. *Nucleic Acids Res.* 31, E119.
- Brandt, O. and J. D. Hoheisel (2004). Peptide nucleic acids on microarrays and other biosensors. *Trends in Biotechnology* 22, 617-622.
- Briones, C., Mateo-Marti, E., Gomez-Rodriguez, C., Parro, V., Roman, E., Martín-Gago, J.A., (2004). Ordered self-assembled monolayers of peptide nucleic acids on gold surfaces with DNA recognition capacity. *Physical Reviews Letters* 93 (20) 208103.
- Briones, C., Mateo-Marti, E., Gomez-Rodriguez, C., Parro, V., Roman, E., Martín-Gago, J.A., (2005). structural and functional characterization of self-assembled monolayers of peptides nucleic acids and its interaction with complementary DNA. *Journal of Catalysis A: Chemical* 228, 131-136.
- Buetow, K.H., Edmonson, M., MacDonald, R., Clifford, R., Yip, P., Kelley, J., Little, D.P., Strausberg, R., Koester, H., Cantor, C.R., Braun, A. (2001). High-throughput development and characterization of a genomewide collection of gene-based single nucleotide polymorphism markers by chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Proc. Natl. Acad. Sci. USA* 98, 581-584.
- Carruso, F., Rodda, E., Furlong, D. N., Niikura, K., and Okahata, Y. (1997). Quartz Crystal Microbalance Study of DNA Immobilization and Hybridization for Nucleic Acid Sensor Development. *Anal. Chem.* 69, 2043-2049.
- Casero, E.; Darder, M.; Diaz, D.J.; Pariente, F.; Martín-Gago, J.A.; Abruna, H.; Lorenzo, E. (2003). XPS and AFM characterization of oligonucleotides immobilized on gold substrates. *Langmuir* 19, 6230-6235.
- Cattani-Scholz, A. Pedone, D. Dubey, M. Neppl, S. Nickel, B. Feulner, P. Schwartz, J. Abstreiter, G. and tornow, M. (2008). Organophosphonate-based PNA-

- functionalization of silicon nanowires for label-free DNA detection. *ACS-Nano* 2, 1653-1660.
- Cattani-Scholz, A. Pedone, D. Blobner, F. Abstreiter, G. Schwartz, J. Tornow, M. and Andruzzi, L. (2009). PNA-PEG modified silicon platforms as functional bio-interfaces for applications in DNA microarrays and biosensors. *Biomolecules* 10, 489-496.
- Chakrabarti, R., and Klibanov, A. M. (2003). Nanocrystals modified PNAs. *J. Am. Chem. Soc.* 125, 12531-12540.
- Corradini, R., and et al. (2004). Enhanced recognition of cystic fibrosis W1282X DNA point mutation by chiral pNA probes by a SPR biosensor. *J. of Mol. Recognit.* 17, 76-84.
- Demidov, V.V.; Potaman, V.N.; Frank-Kamenetskii, M.D.; Egholm, M.; Buchardt, O., Sonnichsen, S.H.; and Nielsen, P.E. (1994). Stability of peptide nucleic acids in human serum and cellular extracts. *Biochem. Pharmacol.* 48, 1310-1313.
- Degefa, T.H.; Kwak, J. (2008). Electrochemical impedance sensing of DNA at PNA self-assembled monolayer. *Journal of Electroanalytical Chemistry* 612, 37-41.
- DeRisi J.L., Iyer, V.R., Brown, P.O. (1997). Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* 278 680-686
- Egholm, M., Buchardt, O., Christensen, L., Behrens, C., Freier, S.M, Driver, D.A., Berg, R.H., Kim, S.K., Norden, B., Nielsen, P.E. (1993). PNA hybridizes to complementary oligonucleotides obeying the Watson-Crick hydrogen-bonding rules. *Nature* 365, 566-568.
- Endo, T., kerman, K., Ngatani, N., Takamura, Y., and E.Tamiya (2005). Label-free detection of peptide nucleic acid-DNA hybridization using localized surface plasmon resonance optical biosensor. *Anal. Chem.* 77, 6976-6984.
- Fabris, L., Dante, M., Braun, G., Lee, S. J., Reich, N. O., Moskovits, M., Nguyen, T.-Q., and Bazan, G. C. (2007). A heterogeneous PNA-based SERS Method for DNA Detection. *J. Am. Chem. Soc.* 129, 6086-6087.
- Fang, B.; Jiao, S.; Li, M.; Qu, Y.; Ximing, J. (2008). Label-free electrochemical detection of DNA using ferrocene-containing cationic polythiophene and PNA probes on nanogold modified electrodes. *Biosensors and Bioelectronics* 23, 1175-1179.
- Fan, Y., Chen, X., Trigg, A. D., Tung, A. D., Kong, J., and Gao, Z. (2007). Detection of MicroRNAs Using Target-Guided Formation of Conducting Polymer Nanowires in Nanogaps *J. Am. Chem. Soc.* 29, 5347-5443.
- Fang, C., Agarwal, A., Buddharaju, K. D., Khalid, N. M., Salim, S. M., Widjaja, E., and Garland, M. V. (2008). DNA Detection using nanostructured SERS substrates with Rhodamine B as Raman label. *Biosens. & Bioelectr.* 24, 216-221.
- Feriotto, G., and al., e. (2001). PNA and biosensors for real time detection of the cystic fibrosis W1282X mutation by SPR. *Lab. invest.* 81, 1415-1427.
- Fodor, S.P.A; Read, J.L.; Pirrung, M.C.; Stryer, L.; Lu, A.T.; Solas, D. (1991). Light-directed spatially addressable parallel chemical synthesis. *Science* 251, 767-773.
- Hashimoto, K., and Ishimori, Y. (2001). Preliminary Evaluation of electrochemical PNA array for detection of single base mismatch mutations. *Lab Chip* 1, 61-63.
- Homola, J. (2003). Present and Future of Surface Plasmon resonance biosensors. *Anal. Bioanal. Chem.* 377, 528-539.
- Höök, F., Rodahl, M., Brzezinski, P., and Kasemo, B. (1998). Energy Dissipation Kinetics for Protein and Antibody-Antigen Adsorption under Shear Oscillation on a Quartz Crystal Microbalance. *Langmuir* 14, 729-734.

- Höök, F., Ray, A., Nordén, B., and Kasemo, B. (2001). Characterization of PNA and DNA Immobilization and Subsequent Hybridization with DNA using Accoustic-Shear-Wave Attenuation Measurements. *Langmuir* 17, 8305-8312.
- Jensen, K. K., and et al. (1997). Kinetics for hybridization of peptide nucleic acids (PNA) with DNA and RNA studied with the BIAcore technique, . *Biochemistry* 36, 5072-5077.
- Kambhampati, D., Nielsen, P. E., and Knoll, W. (2001). Investigating the kinetics of PNA-DNA and PNA-DNA interactions using surface plasmon resonance-enhanced fluorescence spectroscopy. *Biosens. & Bioelectr.* 16, 1109-118.
- Karlsson, R. (2004). *J. Mol. Recogn.* 17, 151-161.
- Katz, E. and Willner, I. (2003). Probing Biomolecular Interactions at Conductive and Semiconductive Surfaces by Impedance Spectroscopy: Routes to Impedimetric Immunosensors, DNA-Sensors, and Enzyme Biosensors. *Electroanalysis* 15, 913-947.
- Kerman, K. Ozkan, D., Kara, P., Erdem, A., Meric, B., Nielsen, P.E., Ozsoz, M., (2003). Label-free bioelectronic detection of point mutation by using peptides nucleic acid probes. *Electroanalysis* 15, 667-670.
- Kerman, K., Vestergaard, M., Nagatani, N., Takamura, Y., Tamiya, E. (2006). Electrochemical Genosensor Based on Peptide Nucleic Acid-Mediated PCR and Asymmetric PCR Techniques: Electrostatic Interactions with a Metal Cation. *Anal. Chem.* 78, 2182-2189.
- Keighley, S. D., Estrela, P., Li, P., and Migliorato, P. (2008). Optimization of label-free DNA detection with electrochemical impedance spectroscopy using PNA probes. *Biosensors & Bioelectronics* 24, 906-911.
- Landegren, U., Nilsson, M; Kwok, P.Y. (1998). Reading Bits of Genetic Information: Methods for Single-Nucleotide Polymorphism Analysis. *Genome Res.* 8, 769-776.
- Lao, A.I.K., Su, X. et al. (2009). SPR study of DNA hybridization with DNA and PNA probes under stringent conditions. *Biosens. & Bioelectr.* 24, 1717-1722.
- Liu, J., Tian, S., Nielsen, P.E., and Knoll, W. (2005). In situ hybridization of PNA/DNA studied label-free by electrochemical impedance spectroscopy *Chem commun*, 23, 2969-2971.
- Liu, J.; Tiefenauer, L.; Tian, S.; Nielsen, P.E.; Knoll, W. (2006). PNA-DNA hybridization study using labeled streptavidin by voltammetry and surface plasmon fluorescence spectroscopy. *Analitycal Chemistry* 78, 470-476.
- Mateo-Marti, E., Briones, C., Roman, E., Briand, E., Pradier, C.M., Martín-Gago, J.A. (2005). Self-Assembled Monolayers of Peptide Nucleic Acids on Gold Surfaces: A Spectroscopic Study. *Langmuir* 21, 9510-9517.
- Mateo-Marti, E., Briones, C., Pradier, C.M., Martín-Gago, J.A. (2007). A DNA biosensor based on Peptide Nucleic Acids on Gold Surfaces. *Biosensors and Bioelectronics* 22, 1926-1932.
- Mantsch, H. H., Chapman, D. *Infrared Spectroscopy of Biomolecules*; Wiley-Liss: New York, 1996.
- Mikkelsen, S.R. (1996). Electrochemical biosensors for DNA sequence detection. *Electroanalysis* 4, 7-14.
- Moulder, J.F., Stickle, W.F., Sobol, P.E. and Bombarn, K.D. *Handbook of X-ray Photoelectron Spectroscopy*, USA, 1992.

- Nielsen, P.E., Egholm, M., Berg, R.H., Buchardt, O. (1991). Sequence selective recognition of DNA by strand displacement with a thymine substituted polyamide. *Science* 254, 1497-1500.
- Orum, H. ; Nielsen, P.; Jorgensen, M.; Larsson, C.; Stanley, C.; and Koch, T. (1995). Sequence specific purification of nucleic acids by PNA controlled hybrid selection. *Biotechniques* 19, 472-480.
- Okahata, Y.; Matsunobu, Y.; Ijiro, K.; Mukae, M.; Murakami, A.; Makino, K. (1992). Hybridization of nucleic acids immobilized on a quartz crystal microbalance *J. Am. Chem. Soc.* 114, 8299-8300.
- Patolsky, F., Katz, E., Bardea, A., Willner, I. (1999). Enzyme-Linked Amplified Electrochemical Sensing of Oligonucleotide-DNA Interactions by Means of the Precipitation of an Insoluble Product and Using Impedance Spectroscopy. *Langmuir* 15, 3703-3706.
- Petrovykh, D.Y., Kimura-Suda, H., Whitman, L.J., and Tarlov, M.J. (2003). Quantitative Analysis and Characterization of DNA Immobilized on Gold. *J. Am. Chem. Soc.* 125, 5219-5226.
- Ramakrishnan, V., M.D'Costa, Ganesh, K. N., and Sastry, M. (2002). PNA-DNA Hybridization at the Air-Water Interface in the Presence of Octadecylamine Langmuir Monolayers. *Langmuir* 18, 6307-6311.
- Reich E., Goldberg H. I., (1964) *Prog. Nucleic Acid Res. Mol. Biol.*, 3, 183-234
- Reisberg, S.; Dang, L.A.; Nguyen, Q.A.; Piro, B.; Noel, V.; Nielsen, P.E.; Le, L.A.; Pham, M.C. (2008). Label-free DNA electrochemical sensor based on a PNA-functionalized conductive polymer. *Talanta* 76, 206-210.
- Sastry, M., Ramakrishnan, V., Pattarkine, M., Gole, A., and Ganesh, K. N. (2000). Hybridization of DNA by sequential Immobilization of Oligonucleotides at the Air-Water Interface. *Langmuir* 16, 9142-9146.
- Steichen, M.; Decrem, Y.; Godfroid, E.; and Buess-Herman, C. (2007). Electrochemical DNA hybridization detection using peptide nucleic acids and $[\text{Ru}(\text{NH}_3)_6]^{3+}$ on gold electrodes. *Biosensors and Bioelectronics* 22, 2237-2243.
- Su, X., Teh, H. F., Aung, K. M. M., Zong, Y., and Gao, Z. (2008). Femtomol SPR detection of DNA-PNA hybridization with the assistance of DNA-guided polyaniline deposition. *Biosensors & Bioelectronics* 23, 1715-1720.
- Ujihara, M., Orbulescu, J. I., T, and Leblanc, R. M. (2005). Film Structures of Poly(Amido Amine) Dendrimers with an Azacrown Core and Long Alkyl Chain Spacers on Water or Ag Nanoparticle Suspension. *Langmuir* 21, 6846-6854.
- Van Benschoten, J.J.; Lewis, J.Y.; Heineman, W.R.; Rosten, D.A.; Kissinger, P.T. (1983). Cyclic voltammetry experiment. *J. Chem. Educ.* 60, 772.
- Wang, J.; Palecek, E.; Nielsen, P. ;Rivas, G.; Cai, X. ; Shiraishi, H.; Dontha, N.; Luo, D.; Farias, P. (1996). Peptide nucleic acid probes for sequence-specific DNA biosensors. *J. Am. Chem. Soc.* 118, 7667-7670.
- Wang, J., Nielsen, P. E., Jiang, M., Cai, X., Fernandes, J. R., Grant, D. H., Ozsoz, M., begleter, A., and Mowat, M. (1997 a). Mismatch-Sensitive Hybridization Detection by Peptide nucleic Acids Immobilized on a Quartz Crystal Microbalance. *Anal. Chem.* 69, 5200-5202.
- Wang, J.; Rivas, G.; Cai, X.; Palecek, E.; Nielsen, P.E.; Shiraishi, H.; Dontha, N.; Luo, D.; Parrado, C.; Chicharro, M.; Farias, P. A.M.; Valera, F.; Grant, D.; Ozsoz, M. and

- Flair, M. (1997 b). DNA electrochemical biosensors for environmental monitoring. A review. *Anal. Chim. Acta* 347, 1-8.
- Wang, J.; Rivas, G.; Cai, X.; Chicharro, M.; Parrado, C.; Dontha, N.; Begleiter, A.; Mowat, M.; Palecek, E.; Nielsen, P.E.; (1997 c). Detection of point mutation in the p53 gene using a peptide nucleic acid biosensor. *Anal. Chim. Acta* 344, 111-118.
- Wang, J. (1998). DNA biosensors based on peptide nucleic acid (PNA) recognition layers. A review. *Biosensors and Bioelectronics* 13, 757-762.
- Wang, S.; Peng, T. Z.; Yang, C. F. (2003). Investigation of the interaction of DNA and actinomycin D by cyclic voltammetry. *J. Electroanal. Chem.* 544, 87-92.
- Wang, J. (2000). From DNA biosensors to gene chips. *Nucleic acid Res.* 28, 3011-3016.
- Will, G., Kudryashov, E., Duggan, E., Fitzmaurice, D., Buckin, V., Waghone, E., and Mukherjee, E. (1999). Excited state complex formation between 3-aminophthalhydrazide and DNA: a fluorescence quenching reaction. *Spectrochim. Acta Part A: Mol Biomol. Spectrosc.* 55, 2711-2717.
- Xu, X. H.; Bard, A. J. (1995). Immobilization and Hybridization of DNA on an Aluminum(III) Alkanebisphosphonate Thin Film with Electrogenenerated Chemiluminescent Detection. *J. Am. Chem. Soc.* 117, 2627-2631.
- Yao, X., Li, X., Toledo, F., Zurita-Lopez, C., Gutova, M., Momand, J., and Zhou, F. (2006). Sub-attomole oligonucleotide and p53 cDNA determinations via a high-resolution surface plasmon resonance combined with oligonucleotide-capped gold nanoparticle signal amplification. *Anal. Biochem.* 354, 220-228.
- Yao, C., Zhu, T., Tang, J., Wu, R., Chen, Q., Chen, M., Zhang, B., Huang, J., and Fu, W. (2008). Hybridization assay of hepatitis B virus by QCM Peptide nucleic acid biosensor. *Biosens. & Bioelectr.* 23, 879-885.

IntechOpen

Intelligent and Biosensors

Edited by Vernon S. Somerset

ISBN 978-953-7619-58-9

Hard cover, 386 pages

Publisher InTech

Published online 01, January, 2010

Published in print edition January, 2010

The use of intelligent sensors have revolutionized the way in which we gather data from the world around us, how we extract useful information from that data, and the manner in which we use the newly obtained information for various operations and decision making. This book is an attempt to highlight the current research in the field of Intelligent and Biosensors, thereby describing state-of-the-art techniques in the field and emerging new technologies, also showcasing some examples and applications.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Eva Mateo-Martí and Claire-Marie Pradier (2010). A Novel Type of Nucleic Acid-based Biosensors: the Use of PNA Probes, Associated with Surface Science and Electrochemical Detection Techniques, Intelligent and Biosensors, Vernon S. Somerset (Ed.), ISBN: 978-953-7619-58-9, InTech, Available from: <http://www.intechopen.com/books/intelligent-and-biosensors/a-novel-type-of-nucleic-acid-based-biosensors-the-use-of-pna-probes-associated-with-surface-science->

INTECH
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2010 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike-3.0 License](https://creativecommons.org/licenses/by-nc-sa/3.0/), which permits use, distribution and reproduction for non-commercial purposes, provided the original is properly cited and derivative works building on this content are distributed under the same license.

IntechOpen

IntechOpen