# 81. Nanoelectronic-Based Detection for Biology and Medicine

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This chapter is a review of the work in nanoelectronic detection of biological molecules and its applications in biology and medicine. About half of the chapter focuses on the methods employed to immobilize deoxyribonucleic acid (DNA) on solid substrates with particular focus on the electronic detection and characterization of DNA. Charge-transfer properties and theories are explained, as such electronic and electrical sensing of molecular-level interactions are very important in medical applications for rapid and cheap diagnosis.

A special tool called nanopore, which has been used extensively to characterize DNA, is then reviewed. A special distinction is made between the characteristics, capabilities, and impacts of the biological and the solid-state nanopores. Nanopores, when used in the ion current measurement setup, are used to measure the behavior of DNA as it traverses the nanopore. When the DNA traverses the pore, the blockage of the ion current is observed as a pulse. The statistical analysis of the pulses yields trends that are used to sort the DNA based on various properties. The nanopores are strong prototypes for biosensors, and have become a major experimental tool for investigating biophysical properties of double and single strands of DNA. The DNA sequence can potentially be determined by measuring how the forces on the DNA molecules, and the ion currents through the nanopore, change as the molecules pass through the nanopore.





## 81.1 Historical Background

Nanotechnology, in the last decade or so, has brought together scientists and engineers from a diverse array of fields to collaborate and share their distinct sets of expertise and tools. The confluence of divergent technologies has provided better handles on nanoscale processes and species. Characterization and control at these fundamental limits have shown immense potential to improve existing technologies and provide better tools for understanding at the most basic levels of nature. This has opened horizons wide open for many diverse areas of research. The major impact it has had, and will continue to have, is in medical applications. Biologists have known for decades that cells and biomolecules are selectively managed, regulated, and controlled, but the tools to organize and direct these interactions are only now emerging, tools to *handle* and

integrate synthetic structures in diagnostics and therapeutics. The nascent tools of nanoscience are helping unravel the physics of biological processes in unprecedented detail. The detection and sensing of biological entities has enormous promise, and challenges of correspondence size. The challenges are manyfold: the interface of the wet salty biological molecules to dry cold solid devices, packaging of the devices so that only the sensing part is exposed to the analytes, faithful translation of the biophysical and biochemical interactions into electrical or optical signals, selectivity of the device against the analyte of interest in the pool of thousands of entities of no interest, identification of the useful signal amongst unwanted noise, sensitivity of the measurement system all the way down to the fundamental limits, and repeatability of measurements. For any system to interrogate the few copies of the analyte of interest reproducibly and reliably, the interface also plays a key role. These challenges are the major impediments to the integration of nanotechnology in biological and medical applications.

A few industries that use a combination of engineering, physics, chemistry, and biology in very nontraditional approaches to develop devices for medical applications have very recently emerged. Some examples are on-chip DNA hybridization and optical detection, DNA detection at the nanoscale for diagnostics in medical, military, and food safety applications, etc. DNA carries the genetic information for living organisms and has a special, important place in many areas such as genetic engineering, drug discovery, and gene therapy. The methods used for immobilizing DNA on solid substrates and the electrical sensing schemes will be detailed in ensuing sections in this chapter. As we go along, we will focus on biological nanopores, also known as ion channels, which are integral parts of the living organisms at various levels. These have inherent capabilities for ion-transport regulation under transmembrane potentials. Ion channels have been used to measure the translocation behavior of DNA as it passes through the channel. These form an important framework for DNA detection, but have inherent issues and problems regarding the stability. Solid-state nanopores, direct analogues of the biological ion channels, are then reviewed. Solid-state nanopores are stable under various environments. Cheap and rapid DNA sequencing using solid-state nanopore channels is the focus of a number of major initiatives, owing to the promise of focused and effective treatment of diseases, and fast genetic analysis in areas such as forensics and legal examination.

## 81.2 Interfacing Biological Molecules

This section is an overview of the techniques used to *probe a single molecule*, i. e., to make a stable and reproducible interface with a biomolecule. It is important to make interface contacts to biological molecules for reproducible and robust interrogation and characterization. Various techniques and chemistries are used to attach biological molecules, such as DNA, to solid substrates, either for conductivity measurements, self-assembly of devices, for bottom-up device fabrications, for patterning matter on the nanometer scale, in biotechnology, and even as biosensors. In the crafty bottom-up paradigm, DNA has played a vital role due to its specific base-pair interactions.

At around the dawn of this century, the major thrust in nanotechnology came from the Moletronics program of the Defense Advanced Research Projects Agency (DARPA) and the National Nanotechnology Initiative (NNI) of the US government. The objective of the Moletronics program is *to develop functioning prototype electronic computer processors and memory integrated on the molecular scale* [81.1]. An important avenue that opened with the vision of Moletronics is towards the development of nanoscale biosensors that are robust, small, accurate, cheap, and have high throughput. A biosensor can be defined as a collection of molecular recognition sites and the transduction components. In case of electrical biosensors, the focus has been on direct measurement of the electrical properties of the molecular interfacing sites. The size of typical organic molecules is in the range of 1–10 nm. To do any realistic electrical measurements through such small objects, we need at least two macroscopic metallic electrodes at the same dimensions.

Self-assembled monolayers (SAMs) of molecules are characterized using a large array of direct analysis tools, but there are few direct label-free char-

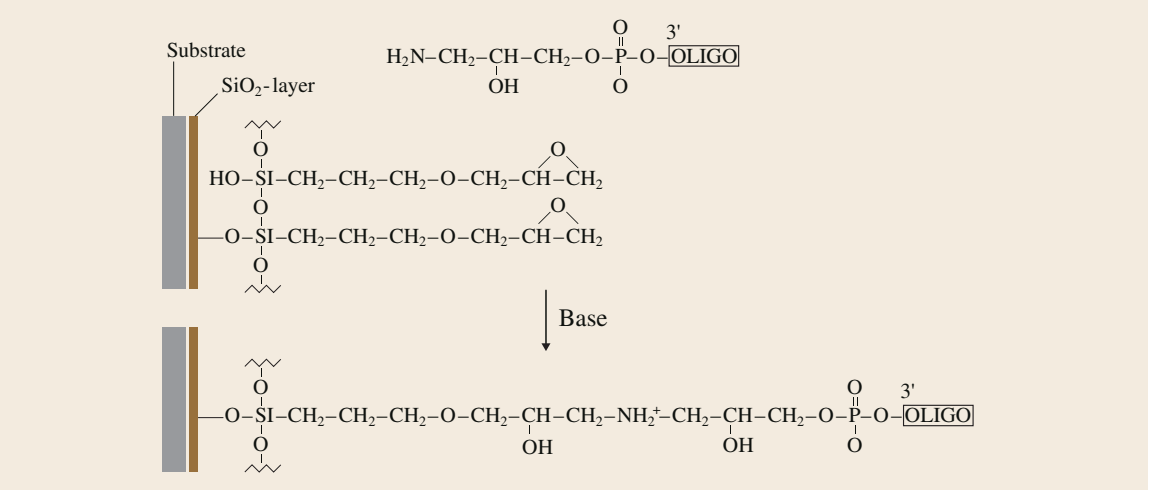


**Fig. 81.2** Schematic depicting chemical bonds in attaching 3′-amino-modified DNA to a thin $SiO_2$ film. Attachment occurs by secondary amine formation between an epoxysilane monolayer and the 3′-amino linkage. From [81.11], by permission of Oxford University Press

cuits, by constructing Ag wires using the self-assembly of a λ-DNA as a template [81.14]. The λ-DNA was uncoiled and stretched to contact two Au electrodes. The $Ag^+$ ions were deposited along the DNA, through $Ag^+/Na^+$ ion exchange as $Ag^+$ formed complexes with the DNA bases, resulting in nanometer-sized metallic Ag aggregates bound to the DNA skeleton. In a similar approach, DNA-templated assembly of Ag clusters has been shown with electrochemical detection [81.8]. The Ag cations were electrostatically *collected* along the gold-surface-tethered DNA duplex. The Ag aggregates were dissolved and the potentiometric stripping of the dissolved Ag was detected on a thick-film carbon electrode.

### 81.2.1 Guidelines for Preparing Silicon Chips for Biofunctionalization

DNA is immobilized by physical or chemical adsorption, the latter being more robust and stable in a variety of ambient conditions. A number of chemistries have been shown by various researchers for attachment of DNA via chemical bonds. The motivation for such attachments has been wide, ranging from biosensors to integrated circuits; for the detection of chemical or biological species in biosensors; to controlled assembly of nanodevices and structures in nanoelectronics and biophysical studies.

The general scheme of DNA attachment is through surface functionalization of the substrate and the modification of DNA with a linker end-terminus, as shown in Fig. 81.3. The linker end-terminus can then bind to a hetero/homobifunctional molecule, or the chemical structure of the linker itself is exploited to react with surface functionalized moieties due to certain binding affinities. The bifunctional molecules have at least two available chemically reactive positions, one of which attaches to the surface-grafted molecular layer and the other to the end-linker of the DNA.

Homobifunctional cross-linkers have two identical reactive groups thus can be used in a one-step chemical cross-linking procedure. DNA modified with various end groups or even without end groups has been shown to attach to certain functional groups. Attachment of ssDNA to the surface molecular SAM is mostly verified

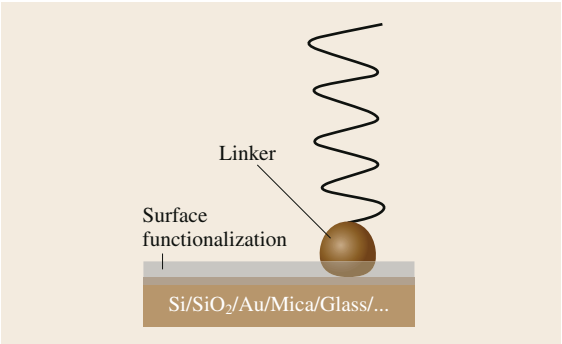


**Fig. 81.3** General schematic of DNA attachment via linker molecule

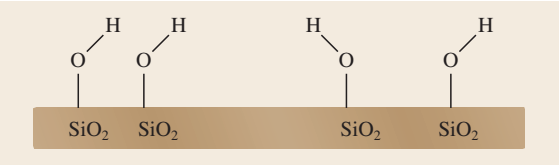


**Fig. 81.4** Hydroxylation of $SiO_2$ surface (after [81.15], by permission of Oxford University Press)

with the fluorescently labeled complementary ssDNA that hybridizes with the adsorbed ssDNA. Other detection schemes involve radioactive dyes such as $^{32}P$, phosphor imaging, and chemiluminescence. We present two protocols that can be adapted for covalent attachment of amine-modified DNA to $SiO_2$ surfaces (adapted from [81.15, 16]).

For direct DNA attachment to the chips, various silanizing agents have been pursued and reported to functionalize $SiO_2$ surfaces. Generally, the silane-containing chemicals have a tendency to react with hydroxyl groups at the surface of the chips, resulting in silanol groups. The hydroxyl group density at the surface of the $SiO_2$ thus influences the self-assembly of the silane layer and ultimately the number of attached DNA. The hydroxyl groups on the surface of $Si/SiO_2$ chips are generally achieved by piranha solution ($H_2O_2 : H_2SO_4$, in various proportions) treatment of the $SiO_2$ surface, or $O_2$ plasma etch treatment. Following these treatments, the OH-rich surface of $SiO_2$ looks as shown in Fig. 81.4.

Exposing the OH-rich surface to a silane results in the reaction of Si of the silanizing chemical with the O group, thus releasing the hydrogen. To directly attach the DNA molecules, an amine modification at the end of DNA can be utilized. The reactive site of the silane (e.g., an epoxide) makes a covalent bond with the amine. The surface coverage of immobilized ssDNA can be verified by using complementary ssDNA tagged with fluorescence. All chip surface processing should be done in a controlled environment, e.g. a glovebox to minimize exposure to ambient moisture and contamination.

For better surface coverage, a homobifunctional linker can be used on top of the surface silane. The chips can be silanized using 3-aminopropyltrimethoxysilane (APTMS), and 1,4-phenylene diisothiocyanate (PDITC) can be used as a homobifunctional agent. Again APTMS hydrolyzes the OH-rich $SiO_2$ surface thus strongly attached through covalent bonds to it, creating a SAM of amino silane anchors. The amine group at the other end of the APTMS covalently attaches with the C of one of the isothiocyanate groups of the PDITC cross-linker. PDITC has two isothiocyanate sites where its C can react with the $NH_2-$ of APTMS. The other isothiocyanate site on PDITC provides amino-reactive terminus for probe DNA attachment. These PDITC-activated chips are thus immersed in the amine-modified DNA, completing the attachment chemistry for the $NH_2$-oligo attachment on the $SiO_2$ surface. The unreacted sites of PDITC are then deactivated by N–N diisopropylethylamine exposure.

### 81.2.2 Verification of Surface Densities of Functional Layers

The functionalized surfaces can be characterized for adsorbed layers by ellipsometry and contact-angle measurements. The silane layer thickness can be measured by ellipsometry. An isotropic value of $n = 1.50$ for the silane layer and $n = 3.858 - 0.018\mathrm{i}$ for the silicon substrate refractive index can be used. The values for monolayers range between 1.45–1.50 in the literature, but this range would give an error of less than 1 Å during measurements. The monolayer thickness can be accurately measured up to $\pm 2$ Å. A number of readings should be taken at different places on the chips and compared with control chips.

The hydrophilicity/hydrophobicity of a surface is usually expressed in terms of wettability. Hydrophilicity and hydrophobicity are general terms used to describe relative affinity of materials for water (hydrogen bonding). Hydrophilicity of a surface is a measure of its strong affinity to the water as the polar surfaces form an H bond with water. The opposite hydrophobic surfaces have aversion to water. Contact-angle measurements with deionized water can show functionalized chips to be less hydrophilic than control chips with only $SiO_2$ and no chemical treatment. The OH-rich surfaces of control chips can make more H bonds with the water and so would be hydrophilic, whereas in relative terms the chemically treated chips would have fewer OH-groups left on the surface and so would be less hydrophilic and can be termed as becoming hydrophobic. A nice example of highly hydrophobic surface is fresh Si surface with no native oxide. Si resists being wetted by water, thus the water forms drops with contact angles $\geq 75°$.

## 81.3 Electrical Characterization of DNA Molecules on Surfaces

In this section we will review studies on charge transfer and direct measurements of electrical conduction through DNA, along with the various charge transport mechanisms that have been proposed and simulated. It has been established that a number of factors and conditions contribute to the behavior of DNA conductivity [81.17, 18]; the number of base pairs (bp), the sequence and length of DNA [81.3], changes in the distance and angle between bases, the influence of water and counterions, humidity, contact chemistry, surface smoothness, electronic contamination, ambient conditions, temperature, and buffer solution components. The double-helix structure of DNA depends on the hydrophobicity of the bases. The hydrophobic bases turn towards the center, away from the water. The presence of the condensed cations counters the negative charges of the phosphate backbone. The water molecules and cations are thus an integral part of the overall picture and exert nonnegligible forces on the base-pair stack [81.19]. All these factors dictate that DNA is a dynamic and very complex system to simulate owing to its structural, chemical, environmental, and vibrational properties.

### 81.3.1 Indirect Measurements of Charge Transfer Through DNA

The idea of DNA being electrically conductive can be traced back as far as 1962, when Eley and Spivey hinted at efficient charge transfer through DNA as *... a DNA molecule might behave as a one-dimensional aromatic crystal and show a $\pi$-electron conductivity down the axis.* They proposed that the DNA structure was ideal for electron/hole transfer proceeding along a one-dimensional pathway constituted by the overlap between $\pi$-orbitals in neighboring base pairs [81.20]. At 400 K, they reported conductivities on the order of $10^{-12}\,(\Omega\,\mathrm{cm})^{-1}$ and energy gaps ($\Delta E$) of about $2.42 \pm 0.05$ eV. While emphasizing lack of knowledge on the ribonucleic acid (RNA) structure at that time, they reported a similar experimental value for RNA. *Snart*, in 1973, reported a similar and reproducible value of the energy gap (2.4 eV) that was affected by ultraviolet (UV) irradiation [81.21]. Snart's measurement method was very similar to the one employed by Eley and Spivey. These can be considered as the starting points of the quest for DNA conductivity.

In the early 1990s, *Barton* and co-workers performed experiments that suggested long-range electron transfer in DNA [81.22]. The donor and acceptor molecules were intercalated on the DNA strands. When the donor was photoexcited, the fluorescence of the donor was quenched due to electron transfer to acceptor. These results showed very rapid transfer of carriers over $> 40$ Å via $\pi$-stacked base pairs. However other researchers disputed these results, as reproduction of the results with other acceptor and donor candidates was found to be problematic. The starting point of the controversy came the very next year when, in similar experiments, *Brun* and *Harriman* used organic donors and acceptors [81.23] and concluded that charge transfer rates drop off quickly with increasing length of the DNA. *Ly* et al. studied the mechanism and distance dependence of radical anion and cation migration and suggested that the structural flexibility of DNA dictates mixed behavior of hole-hopping and continuous orbital mechanism [81.24]. Such a mechanism in which the injection of charge disturbs the molecular structure is called a phonon-assisted polaron-like hopping mechanism. Such a disturbance in DNA most likely results in the reduction of intrabase distance and unwinding of DNA, giving way to increased $\pi$-electron overlap and shift of internal charges inside H bonds. *Giese* and *Wessely*, in 2000, verified these two mechanisms experimentally [81.25]. They reported a coherent superexchange reaction (single-step tunneling) and a thermally induced hopping process for long-range charge transfer, slightly influenced by the number of intercalated adenine–thymine (AT) bases. Generally, in a hopping mechanism, the guanine (G) base is considered the most favorable for landing of holes or trapping, basically because it has the least ionization potential among the four bases: G < A < cytosine (C) < T, independent of nearest-neighbor effects. Since then, more experimental and theoretical research has resulted in seemingly contradictory results.

### 81.3.2 Direct Measurement of DNA Conduction and DNA Conductivity Models

The sensing of DNA has been a direct result of the electrical characterization studies. DNA has been shown to have metallic-like conductivity [81.26], semiconducting behavior [81.27], and also as an insulator [81.28].

*Fink* and *Schonenberger* used gold-coated perforated carbon foil as a sample holder to make DNA

and about 1 $Å^{-1}$ for coherent tunneling by quantitative analysis using kinetic rate equations [81.46].

The mechanisms proposed in the theoretical models may or may not occur in a system altogether, but there is higher probability for presence of more than one mechanism in any given experiment. The ideal model should take care of the effects of DNA structure, thermal motion of charges as described by classical models, effects of cations in the solution on the structure and on the Coulomb charging, temperature, intermolecular and intramolecular attractions and repulsions, effect of contacting conductors and the chemistries used to contact the DNA, etc. Almost all studies consider charge transport and conductivity on the path along the DNA length. Little, if any work has been done, until lately, on charge transport in the direction perpendicular to the axis of the DNA backbone. *Zwolak* and *Di Ventra* described unique signatures of each base due to their differing electronic and chemical structures [81.47]. They considered a system of electrodes through which a ssDNA passes such that at any given time only one base interacts with the electrodes. Such a system can be visualized as a nanopore made with a very thin membrane, with electrodes on the very edges. With electrode–electrode spacing of 15 Å, they found the ratio of current of A ($I_A$) and the current of other bases ($I_X$) to be $I_A/I_X = 20$, 40, and 660 for X = G, C, and T, respectively. This difference in ratios is postulated to stem from relative positions of the Fermi level with respect to HOMO and LUMO, and the density of states at Fermi level. The effects of nearest neighbor were also calculated, with T found to be the most sensitive to such effects. These findings are very interesting as they can be used in conjunction with nanopore measurements of DNA translocation, as will be explained in next section.

## 81.4 Nanopore Sensors for Characterization of Single DNA Molecules

Biological entities, such as cells, proteins, and DNA, carry charges, and can be forced to move by an electric field in a buffer solution, a phenomenon called electrophoresis. Electrophoresis is usually applied in a gel or capillary to separate biological entities based on their size and charge. Electrophoresis in gel and capillaries has given rise to the idea of characterization by a single pore through which charged entities can be driven. Therefore, characterizations of biological entities by a single pore are actually an analogous application of gel and capillary electrophoresis. The basic design of a nanopore characterization setup consists of two compartments, filled with saline buffer solution, separated by a membrane with the pore, with an anode and a cathode set in each compartment; the pore provides the only path for ionic currents and the electrophoretic movement of DNA or other biological species of interest. When DNA moves electrophoretically from the cathode to the anode, it traverses the pore. As it does so, the ionic current is altered and most typically decreases due to pore blockade. When DNA has passed through the pore, the current recovers to its original level. The characteristics of DNA can possibly be determined from these current fluctuations.

### 81.4.1 Biological Nanopores

*Kasianowicz* et al. pioneered the use of a single 2.6 nm diameter $\alpha$-hemolysin ($\alpha$-HL) channel [81.48]. $\alpha$-HL is a protein toxin from the bacterial species *Staphylococcus aureus*. A solvent-free bilayer membrane of diphytanoyl phosphatidylcholine (an artificial lipid bilayer membrane) was formed across a $\approx 0.1$ mm diameter orifice. The orifice was in Teflon partition separating two buffer-filled compartments. When $\alpha$-HL was added to the compartment, it reconstituted into the lipid bilayer, making a channel. A single channel usually formed within 5 min. They used these channels for current fluctuation detection when a single DNA traversed and passed through the pore. The translocation times, or the pulse widths, were proportional to the DNA lengths. A patch clamp amplifier was used to convert current to voltage. In the absence of DNA, applying a potential of $-120$ mV resulted in ionic currents free of pulses. Following the addition of DNA to the *cis* side of the protein pore, numerous short-lived current blockades occurred (the current was reduced by 85–100%). The blockades lasted from several hundred to several thousand microseconds, depending on the polymer length. For a given polymer length, the total number of pulses was seen to be directly proportional to polymer molar concentration. It was further shown that DNA length, one of the DNA characteristics, was directly proportional to the mean lifetime of the peaks in the signals. This realized determination of lengths of individual RNA or DNA chains using single-channel measurements. Applying the same principle towards a chemical sensor, *Bayley* and co-workers detected or-

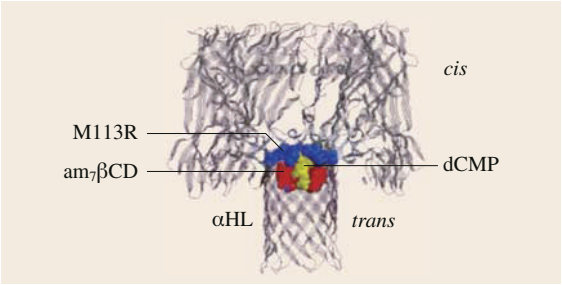


**Fig. 81.7** Schematic depicting the $\alpha$-HL pore, the met-113 substituted with Arg (*blue*), $am_7\beta CD$, and the 2′-deoxycytidine 5′-monophosphate molecule (dCMP). Adapted with permission from [81.52]. Copyright 2006 American Chemical Society

tained by lifetime histogram analysis and it was seen that the type of mismatched base pair influenced the lifetime, and that the mismatch had the most dramatic effect when it was positioned in the middle of the oligonucleotide. Using an array of DNA tethered $\alpha$-HL pores they sequenced a complete codon in an individual DNA strand. They did not look into any change in the translocation time of a DNA sequence complementary to the tethered DNA as compared with the translocation of the same through an untethered pore. In another report, *Bayley* and co-workers reported another engineered $\alpha$-HL pore approach to detect individual nucleoside monophosphates (Fig. 81.7) [81.52]. The nucleoside monophosphates are individual bases of DNA (A, G, C or T) with a single phosphate group. The nucleoside monophosphates, like DNA, are also negatively charged thus traverse the pore under the trans-channel bias [81.53]. The $\alpha$-HL pores were modified with amino-cyclodextrin $am_7\beta CD$ as *adapter* and its positive charges altered the translocation time for the nucleosides. They attained accuracy as high as 98% (for G) using this approach.

### 81.4.2 Solid-State Nanopore

Researchers have been making *point contacts* since as far back as 1977. These were used mainly for material science studies, especially ballistic transport experiments. The schematics of these structures were very much like nanopores, as these stand today, but the aim was quite different [81.54, 55]. The design of current solid-state nanopores was inspired by one such work by *Gibrov* et al. [81.55] and these are the next most ideal replacement for biological nanopores owing to several established and foreseen advantages. First, as for other chemical sensors, sensitive electronic circuitry and photonic sensing capabilities can be integrated directly into a pore–membrane system. Secondly, simultaneous and automated analysis of hundreds of arrays of different channels can potentially be achieved with such an integrated system. Next, they are more robust to withstand wide range of temperature, analyte solution properties, environments, and chemical treatments that might be required for target detection and to eliminate interference. Finally, these can be customized to fit in practical biosensors. These properties have heightened the interest in solid-state nanopores, with particular attention as progenitors of rapid and cheap next-generation DNA sequencing *machines*.

In an earlier report on solid-state nanopores, *Li* et al. utilized a feedback-controlled ion-beam sculpting process to make a nanopore in a silicon nitride membrane [81.56]. A bowl-shaped cavity was made in a silicon nitride membrane and the material was removed from the other side of this membrane using Ar ion-beam sputtering. As soon as the ion-sculpted side reached the bottom of the cavity a hole around 60 nm was opened. Continuous ion-beam exposure reduced the hole to 1.8 nm diameter and ultimately closed it. Two mechanisms were proposed to account for the pore size reduction: surface matter moving due to reduced viscosity to relax the stress caused by implantation, or the creation of adatoms on the surface by incident ions that could diffuse to close the pore. A 500 bp dsDNA translocation experiment was done with a 5 nm diameter pore. Comparative translocation measurements on 3000 and 10 000 bp dsDNA with 3 and 10 nm pores were also reported [81.57]. Longer DNA translocation was noted to be more complex because of folding.

Dekker's group reported the fabrication of a nanopore with electron-beam lithography EBL and transmission electron microscope TEM techniques [81.3]. Their method realized an in situ observation of pore size while the size was precisely controlled at the nanometer scale. *Chang* et al. reported a similar approach, although developed independently [81.58]. They fabricated 50–60 nm long 4–5 nm diameter nanopore channels (NPC), in micromachined Si membranes. The NPCs were fabricated in a double-polished silicon-on-insulator (SOI) wafers. They used EBL to initially define the pore and fabricated 3–4 nm nanopores with standard solid-state processing. The pore was examined visually by using TEM while its diameter shrank to the desired size. The pore shrinkage occurred under an electron beam of high energy. However, their mechanism was completely different from Li's process. In this

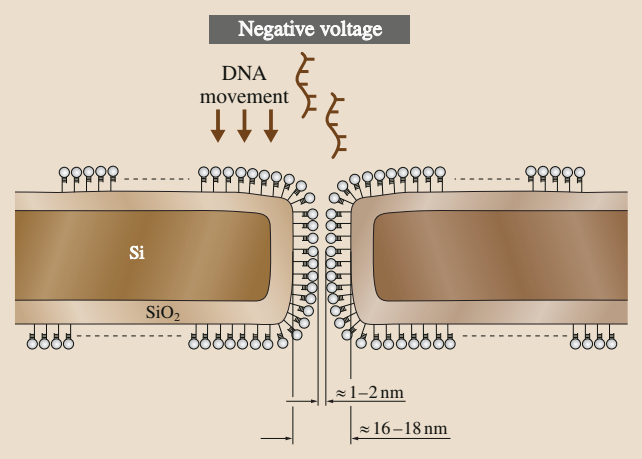


**Fig. 81.9** Schematic of the functionalized nanopore channels. The chemical modification and the probe hairpin-loop DNA reduced the NPC diameter. Adapted by permission from Macmillan Publishers Ltd: Nanotechnology [81.77], copyright (2007)

DNA midway through the pore and force measurements were carried out. The study reported effective charge of $0.50 \pm 0.05$ electrons per base pair, which is equivalent to 25% of the bare DNA charge.

As explained earlier, it is important to slow down DNA to gather more information with reasonable bandwidth requirements on the electronic measurement systems, while still maintaining high signal-to-noise ratio. In a recent report, *Iqbal* et al. chemically functionalized the nanopore channels (NPC) with hairpin-loop DNA [81.77]. The functionalization with known hairpin-loop *probe* ssDNA showed selective transport behavior for *target* ssDNA that was complementary to the probe. This chemical modification of the surface also restricted the movement of the target ssDNA. The hairpin-loop configuration imparted single-base-mismatch sensitivity. Such a single base mismatch between the probe and the target resulted in longer translocation pulses and a significantly reduced number of translocation events. Figure 81.9 shows a schematic of the functionalized NPC and the effective diameter of the NPC after functionalization. They used short ssDNA as target molecules. The target ssDNA were stiff polymers at the length used in this study, thus avoiding the *folding* effect [81.78]. These single-molecule measurements allowed separate measurements of the molecular flux and the pulse duration, providing a tool to gain fundamental insight into the channel–molecule interactions. They discovered that perfectly complementary ssDNA translocated faster and was transported in higher numbers across the pores compared with a target that had even a single base mismatch with the probe molecules. The experimental observations showed that nanopores can be used as selective sensors for genes of interest and that such selectivity could be electrically measured by the translocation signatures at the single-molecule level.

### 81.4.3 The Promise of Low-Cost DNA Sequencing

The human genome project, completed in 2003, is estimated to have cost US$ 2.7 billion [81.79]. It took more than 12 years to complete. With the advent of novel sequencing technologies and abundant computation power the cost and time needed for sequencing a genome has reduced dramatically, now at $\approx 3$ cents/base [81.80]. However, this is an enormous amount for 3 billion bases to be sequenced. Nanopores, in spite of their shortcomings and open challenges, are a hope to achieve the US$ 1000/genome target as set by the National Institutes of Health with more than US$ 70 million in grant monies. The challenges of nanopore technology are both material and electrical. The reproducibility of the nanopore channels is a concern where even an angstromical difference in channel length changes the conductivity of the pore and hence the baseline currents. Although the baseline can be normalized, the noise varies from pore to pore owing to the differences of physical and chemical properties of the pore wall surfaces. There are also issues of bandwidth; DNA passes way too fast for the electronics readout to capture the full details of translocation. The best optimal bandwidth of measurements would still miss too many fast translocation events, significantly skewing the analysis of the pulse behavior, while reducing the noises. The bandwidth issue thus limits the speed of classifications that can be made with nanopores. There are a number of noise sources that are encountered in nanopore measurements; especially $1/f$ (flicker) noise. Tackling such diverse scientific challenges in the nanopore measurements is an uphill task. However, the promise of cheap and fast sequencing machines makes every effort worth pursuing.

## 81.5 Conclusions and Outlook

This chapter summarized the state of the art in probing the electrical properties of molecules with special emphasis on DNA conductivity. The various techniques for DNA immobilization were discussed. The biological ion channel pores extended to the idea of solid-state nanopore, and the state of the art in nanopore fabrication and its applications, were covered. Very recently, two review articles have appeared [81.81, 82] that cover the history and state of the art of the nanopore research in more detail. For details of laboratory protocols of setting up biological and synthetic nanopores for DNA analysis, a laboratory manual appeared in December 2007 [81.83]. It is the consensus that nanopores have set some ambitious goals for the research community, foremost being the sequencing of the human genome for less than US$ 1000 dollar. The confluence of the sophisticated electronic sensing and nanopore detection techniques has strong potential of achieving the US$ 1000 genome and much more.

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