DNA and ion transport through solid-state nanopores

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Chapter 1

Introduction

This chapter provides a general introduction of nanopore research by discussing the broader research areas which encompass it. We sequentially illustrate the fields of biophysics, nanotechnology, and nanobiotechnology, before turning to nanopores. We exemplify the interaction between physics and biology by giving examples of the birth and development of two classic biophysical research areas. The field of nanotechnology is introduced by discussing its origin, basic technologies, and examples of current achievements. Biophysics and nanotechnology meet in a research area classified as nanobiotechnology, and we highlight some spectacular studies. Finally, we present an overview of the nanopore research field. We discuss its origin, two appealing examples of pores in biology, the experiments performed on biological nanopores, and the appearance of solid-state nanopores. We finish this chapter by an overview of the content of this thesis.
1.1 Small scale riddles

When asked about my profession by friends and acquaintances with no particular involvement in science, I respond by posing a riddle

‘I work with small holes,
I sometimes wear a white outfit,
and I am not a dentist’.

As expected, they can not solve the riddle and it usually merely results in frowning. So let’s explain the riddle. Since 2003 I have been doing research in the field of biophysics, and nanobiotechnology. This field studies phenomena in the biological cell by using tools which allow sensing and manipulation on the nano-scale. Our world of thought might be grasped by the concept displayed in the 1980 animation movie ‘The Wonderful Adventures of Nils’. Here a 14 year old farm boy named Nils Holgersson, is shrunk down to a size of a thumb, experiencing a totally different world in which a crumb of bread serves as an entire meal, people appear as giants, and a goose’s neck is a comfortable place to travel across Sweden. The world we explore requires shrinking ourselves to the nano-scale, $10^8$ times smaller than the unfortunate Nils. Here we experience the inside of a living cell as the midst of a bustling city. We can see transportation tracks, energy factories, waste disposal systems, communication lines, and a complex version of the polder model. In the following I will introduce the world of nanobiotechnology, by discussing biophysics and nanotechnology and their merging. Subsequently, I will focus on nanopore research, and finally I will present an overview of the contents of this thesis.

1.2 Biophysics

The physical sciences have always had a large impact on biology through the development of imaging devices and techniques. Antonie van Leeuwenhoek (1632 – 1723) for instance, a Dutch tradesman and scientist from Delft, improved the optical microscope, and became the first person to see bacteria. X-ray and magnetic resonance imaging (MRI) are current examples of familiar techniques employed in hospitals. These imaging techniques are also used to resolve protein structures and conduct brain research, respectively. The interaction between biology and physics is however not limited to improvements of physical instrumentation. This is not very surprising, as can be illustrated with the words of one of the early biophysicists, the German Emil du Bios-Reymond (1818-1896) [1]:
'the more one advances [...] the more one will have reasons for ceasing to believe that the phenomena of life are essentially different from physical phenomena.'

The behavior of all components inside a living organisms are bound to the same physical laws as established for lifeless matter. The task of explaining phenomena encountered in biology with these laws involves many areas of physics, as phrased by Max Delbrück (1906 − 1981) [2]:

'In order to apply physics successfully in biology you have to know more physics than you have to know to do physics, not less but more, [...] since so many aspects of physics are involved in the simplest biological phenomenon. [...] You get involved with everything – with motility, and with diffusion, and with viscosity, and with hydrodynamics, and with electrical phenomena and electrochemistry and so on.'

Physicists are trained to solve problems using a quantitative and analytical approach, and this spirit has enriched the study of biology. Below, I will illustrate the field of biophysics by giving examples of the birth and development of two research areas in which biology as well as physics played a huge part.

Luigi Galvani (1737 − 1789) and Alessandro Volta (1745 − 1827) discovered in 1771 that they could make muscles of dead frogs twitch when using electrical sparks (see Fig. 1.1(a)). Even when legs were removed from the frogs body they jumped in response to static charges. The effect, was first denoted as 'animal electricity' by Galvani. His reports were allegedly read by Mary Shelley (1797 − 1851) providing inspiration for the well known novel Frankenstein, and they also attracted the attention of many physicists. Research conducted on the phenomenon finally resulted in a basic understanding of the electric signal conductance along nerve cells. Alan Hodgkin (1914 − 1988) and Andrew Huxley (1917) described the principles of operation in a mathematical form in 1952. They hypothesized the existence of small ion channels in cell membranes which regulate the in- and outward flux of ions, and as a result produce a membrane potential. These small channels were discovered in 1972, by Bernard Katz (1911 − 2003) and Ricardo Millesi (1927). Nowadays, a wide variety of ion channels have been discovered. Their gates open and close for the passage of ions depending on different stimuli, such as the local potential and the binding of specific molecules to recognition sites. Ion channels are still the subject of intense biophysical research.
Figure 1.1: (a) Details of Galvani’s laboratory [3] (b) Structure of DNA as originally sketched by Watson and Crick [4]. The two ribbons symbolize the phosphate-sugar chains and the horizontal rods the pairing between the bases.

today.

In the second example, the understanding of heredity, the interplay between biology and physics will lead to the currently most-well known discovery made in biophysics. Gregor Mendel (1822 – 1884), a priest and scientist now known as ‘the father of genetics’, pioneered the study of heredity. Mendel was heavily influenced by the physical sciences and appreciated quantitative approaches. His work was not widely accepted until after he died, and the fundamental questions on the physical basis of heredity were still unresolved. Max Delbrück (1906 – 1981), trained as a theoretical physicist in Göttingen, had a large interest in these fundamental questions and converted to biology. He founded the ‘phage’ school, an informal network of physicists and biologists studying the origins of heredity. The group studied the effects of bacteria-infecting viruses (bacteriophages) on bacteria. In 1953 this line of research finally lead to the structure of deoxyribonucleic acid (DNA) as a double-helix (see Fig. 1.1(b)) by James Watson (1928) and Francis Crick (1916 – 2004). The detailed physical principles at the most basic atomic level underlying heredity were discovered soon after.

The two examples given above illustrate the mutual interaction between biology and physics. Today, the tango of the biological and physical sciences is getting even more intimate, as advances yield both more information and new questions.
1.3 Nanotechnology

The attention for nanotechnology has grown considerably in recent years. Well-known commercial products use the term 'nano' for branding, such as the iPod nano and the Tata nano, and nanotechnology novels fantasize on future possibilities and threats, as in the novel Prey by Michael Crichton. The word nano is derived from the Greek nanos, which means dwarf. It is used as a prefix in the metric system to indicate length scales of one billionth of a meter \(10^{-9}\) m, the nanometer (nm). Nanotechnology can be loosely defined as the fabrication, utilization and control of structures on a size scale of 100 nm or smaller. In the following I will briefly characterize the field of nanotechnology by discussing its origin, basic technologies, and the current discoveries.

Richard Feynman (1918 – 1988) gave birth to the field of nanotechnology in 1959 by his classic speech 'There’s plenty of room at the bottom'. Here, he describes a new field of research associated with manipulating things on a small scale. He emphasizes that miniaturization can in principle be pushed to much smaller size scales (top-down) and introduces the bottom-up approach [5]:

'But I am not afraid to consider the final question as to whether, ultimately—in the great future—we can arrange atoms the way we want; the very atoms, all the way down!'

Apart from merely 'the fun of it', he points out some expected benefits from the ability to manipulate and control things on a small scale. The fabrication of small scale machines would reduce the use of materials and the associated costs, and since information needs time to travel, smaller computer chips will be able to work faster. Moreover, the bottom-up approach could ultimately result in the synthesis of any chemical substance by putting the atoms in the right position. Feynman also points out that on such small scales weight and inertia are of relatively no importance, materials might stick together by molecular (Van der Waals) attractions, and the laws of quantum mechanics come into play.

To control and investigate matter on a small size scale it is necessary to be able to position, move and measure things on this scale. This became possible with the invention of the scanning tunneling microscope (STM, in 1981) in which a very sharp tip, the probe, is positioned and moved over a sample with 0.1 nm resolution [6]. The developed technique compares well to the reading of braille by blind people, with the dots now representing single atoms. The STM is a diverse tool as it does not only allows for measurements on individual atoms, but it can also be used to reposition atoms (see Fig. 1.2(a) and its accompanying
text below). The invention resulted in the development of whole class of different scanning probe microscopes (SPMs) and launched nanotechnology research. Other important techniques include developments in semiconductor fabrication, chemical synthesis, and optical microscopy.

The vision of Richard Feynman has provided a roadmap for nanotechnology research. With the use of the scanning tunneling microscope it is indeed possible to fabricate structures by manipulating the very basic building blocks of matter, the atoms [7]. An example is given by the ‘carbon monoxide man’ shown in Fig. 1.2(a). Furthermore, the STM can be used to establish particular chemical bonds as demonstrated by the achieved controlled bond formation between carbon monoxide (CO) and an individual iron atom (Fe), resulting in Fe(CO) [8]. Apart from structures fabricated using the STM, molecular-sized synthetic motors can be build by chemical assembly. Examples include controlled molecular rotors and opening and closing nanovalves [9]. Recently, this research lead to the re-invention of the wheel, now rolling at the atomic scale [10] as shown in Fig. 1.2(b). Complementing these bottom-up created structures, top-down fabrication has also resulted in impressive achievements in recent years. Figure 1.2(c) shows a microelectromechanical system (MEMS) developed on a chip. These MEMS sense the environment, process the information, and act accordingly. Nowadays, also NEMS are being developed: nanoelectromechanical systems. In addition to the research on these integrated systems, new electrical circuit elements are fabricated using only a single current-carrying electron. Individual electrons behave according to the laws of quantum mechanics and this offers new design opportunities. Examples include computation based on quantum mechanical phenomena (the quantum computer) [11], and already commercially available gaint-magnetoresistance (GMR) based computer hard-drives [12, 13]. Existing basic electronic elements such as the transistor, the fundamental building block of computer chips, have been fabricated with a single-molecule as the key element [14]. Despite the huge progress made in nanotechnology, it is currently mainly a research activity. Some applications like sunscreens and paints use nanomaterials, but its true potential still remains to be harnessed.

1.4 Nanobiotechnology

We now return to the nanometer dwarf-size to have a look at the processes inside of a living cell. Here, we find a continuous activity of molecular complexes which regulate the in- and outward transport of membrane enveloped compartments, correct errors emerging in the DNA genetic code, and disassemble foreign
1.4 Nanobiotechnology

Figure 1.2: (a) The ‘carbon monoxide man’ consists of 28 carbon monoxide molecules on platinum and measures 4.5 nm from head to foot [15]. (b) Calculated configuration of a single-molecule structure with two wheels of 0.8 nm diameter. The rotation of the wheels is indicated by arrows [10]. (c) Spider mite on a MEMS chip [16]

invaders such as viruses, to name a few. Feynman’s lecture on manipulating and controlling things on a small scale was inspired by biology [5]:

'A biological system can be exceedingly small. Many cells are very tiny, but they are very active; they manufacture various substances; they walk around; they wiggle; and they do all kinds of marvelous things—all on a very small scale. Also, they store information. Consider the possibility that we too can make things very small which does what we want—that we can manufacture an object that maneuvers at that level!'

The tools and developments in nanotechnology can now be used to study the individual elements encountered in biology. Here biophysics and nanotechnology meet and the resulting research is classified as nanobio- or bionanotechnology.

Biomolecules are studied either directly inside (in vivo) or outside (in vitro) of the cell. In vivo studies generally rely on the binding of a marker molecule specifically to the molecule of interest, after which it can be imaged using optical techniques [17–20]. A spectacular example is shown in Fig. 1.3(a) which shows microtubule bundles attached to chromosomal DNA during cell division. In contrast, in vitro studies extract the molecule(s) of interest from the cell and study their behavior in a controlled environment. Two important techniques used in these studies are the atomic force microscope (AFM), a scanning probe microscope especially suited to image non-conducting biological material, and magnetic and optical tweezers [21, 22]. The tweezers can be used to exert and measure forces on a micrometer-sized bead attached to a single biomolecule. An individual DNA molecule, for instance, can be bound between a surface and the
Apart from the study of biological elements in- and outside of the cell, biological structures can also be used as building elements in nanotechnology. Examples include the use of the unique assembly properties of DNA to build intricate structures towards a biochip computer [23], and the use of molecular motor complexes from cells for directed transport on chips [24], as displayed in Fig. 1.3(b). Another large effort is the fabrication of nanostructured devices to study and analyze properties of biomolecules, an example of such a device is shown in Fig. 1.3(c) and (d). Other examples include solid-state devices for the separation of DNA strands of different lengths [25, 26], and the research towards a DNA sequencing device capable of determining the DNA code [27].
1.5 Nanopores

We now turn to the subject of this thesis: nanometer-sized pores (nanopores), or more specifically DNA and ion transport through solid-state nanopores. We fabricate small pores, much like the biological ion channels discovered by Katz and Miledi, using tools from nanotechnology. An individual nanopore is placed in between two liquid reservoirs, forming the only connection between them. The transport of ions and DNA through the nanopore is studied by detecting ionic currents through the nanometer-sized pore. In this section I will give an overview of this field of research by discussing the coulter counter, examples of pores in biology, the use of biological pores, and finally the subject of this thesis: the use of solid-state nanopores.

1.5.1 The Coulter counter

The coulter counter is the predecessor of solid-state nanopores. Here, I will explain its development and detection scheme, a simply principle that is also used in solid-state nanopores for single-molecule detection. Coulter detectors are used for the counting of cells. This can help diagnose anemia and other conditions affecting red blood cells. In the past counting was done manually under a microscope, a time consuming method which rarely produced repeatable results.

Wallace H. Coulter (1913−1998) and his brother Joseph R. Coulter (1924−1995) joined forces in 1946 to develop an automated cell counting machine. Their initial idea was based on a concept described by Moldavan, in which cells, forced by pressure through a capillary glass tube, block a light beam [30]. Individual cells would then be registered automatically using a photoelectric apparatus. However, the results were not encouraging. The brothers recognized that the passage of cells through an electrolyte-filled capillary might cause a modulation of the electrical current through the hole. During the passage of a cell the current would be temporarily blocked, as part of the liquid volume carrying the current is now occupied by the cell. Figure 1.4 shows a schematic representation of the detection scheme. The Coulter brothers initially measured electrical contrast between cells and their ionic suspension medium of about 10 times the signals obtained photoelectrically, allowing for accurate cell counting. This new simple method was difficult to patent, as expressed by the response of several attorneys [31]:

'You can't patent a hole'

Nevertheless, a patent was finally issued in 1953. In 1954 Wallace and Joseph Coulter introduced the Coulter Counter® Model A, the first automated blood
cell counter. Their invention formed the basis of a thriving industry.

The simple principle, the detection of micrometer-sized cells by their induced temporal current blockade in a small opening, could now be extended to smaller analytes. As the size of the opening dictates the size of analyte that is detectable, fabrication of small pores is crucial. In 1970 the track-etched method was used to provide sub-micrometer cone-like openings in a thin foil. It uses an irradiation source to affect the atomic structure of the foil along a track, which can subsequently be removed by chemical etching. Use of these pores resulted in the detection of 60 nm particles [32]. The use of even smaller pores, with nanometer-sized openings, would extend the coulter counter concept even further and enable detection and counting of single molecules.

1.5.2 Biological nanopores

The cell has a wide variety of small pores to be able to communicate with the world outside of membrane bound compartments. These channels open and close under influence of specific stimuli, and more intricate transport mechanism such as pumps also exist [34]. Due to their small size, these biological pores are ideal candidates to extend the coulter counter concept to the detection of single molecules. First, I will discuss two attractive examples of biological pores.

The governing of a cell is mainly practised inside the cell nucleus, coordinating for example growth and reproduction. It consist of a membrane-enclosed compartment containing the DNA and many proteins. Transport from the nucleus into the exterior cytoplasm and vice versa is crucial for gene expression and DNA repair, and necessary for survival. The membrane of the nucleus has a unique

![Figure 1.4: Schematic of the detection principle used in conventional Coulter counters. An external pressure difference drives micrometer-sized particles through a glass partition. Each particle passing the opening can be detected as a temporal conductance blockade [33].](image)
1.5 Nanopores

pore to communicate with the cell’s cytoplasm: the nuclear pore complex (NPC). The pore has an approximate inner and outer diameter of 40 and 100 nm, respectively [35]. It contains flexible unstructured proteins in its interior, possibly important in regulating the passage of molecules. Small molecules simply diffuse through these pores, whereas larger molecules are only exported or imported carrying an appropriate tag [36]. Many details of the transport through these structures are still unresolved.

Bacteriophage T4, a member of the bacteria-infecting viruses studied by Max Delbrück, consist of an dome-shaped head domain containing the DNA (approximately 86 by 120 nm diameter) [37], a tail domain (22 nm in diameter and 98 nm long) [38], and a baseplate (52 nm diameter and 27 nm high) [39], see Fig. 1.5(a). Characteristic long and short tail fibers are attached to the baseplate. In contrast to many other viruses, bacteriophages enter the host cell only with their genome, whereas the capsid remains outside of the cell. Upon binding of the fibers to receptors at the bacterial-cell outer membrane, the baseplate changes its conformation. As a result, the tail domain contracts and protrudes to pierce the cell membranes of the bacterium. Subsequently, the viral DNA is injected through the small opening [40, 41].

I now turn to the biological protein pore α-hemolysin, a toxin used by the bacterium Staphylococcus aureus in its struggle for resources. Upon secretion of α-hemolysin monomers, they assemble to form a transmembrane pore in susceptible cells. The pore acts as a toxin as its insertion results in the loss of vital molecules, ionic gradients and osmotic swelling. This can finally lead to cell wall rupture and dead of the host cell. The α-hemolysin protein pore has due to its stability and size, become the protein of choice to detect the passage of single molecules. The protein structure is a 10 nm long mushroom-shape of which one half, on the side denoted cis, is exterior to the membrane and the other half, at the transside, transpierces the membrane, see Fig. 1.5(b). The limiting apertures are 2.6, 4.6, 1.5, and 2 nm going from the cis to the trans side [42], and the pore contains many charged amino acids at its surface. Experiments start with the formation of a bilayer lipid membrane on a small \(\approx 25 \mu m\) diameter aperture in between two liquid reservoirs containing salty solutions. Subsequently, protein pores are added to a single liquid compartment and a sudden current increase is detected upon spontaneous assembly of a single pore into the membrane. The compartment containing the protein pores is now flushed to prevent multiple insertions. The feasibility of the single-molecule coulter-counter concept was first proven by Bezrukov, who detected the diffusive passage of single polymer molecules (as small as 0.5-1.5 nm) through the protein pore [43]. Kasianowicz was the first to show that the passage of DNA through α-hemolysin results in
clear temporal current blockades [44]. Whereas for the coulter counters pressure is used to force cells through an aperture, here the applied electrical field acts on the charged DNA and drives it through the nanopore.

Having extended the coulter counter to the single-molecule level, research advanced to determine characteristic details of the passing molecules [45, 46]. Different RNA molecules, a nucleic acid closely related to DNA, of 100 identical bases each were found to cause different current blockade signals [47, 48]. Even single-nucleotide differences were detected from specifically engineered hairpin DNA structures [49]. The experiments performed on the biological \(\alpha\)-hemolysin protein pore show great prospects to use 'a small hole' as a sensor for single (bio)molecules.

1.5.3 Solid-state nanopores

Preferably, one would be able to use stable individual nanopores of any given dimension and surface characteristic. In addition, straightforward integration into fabricated devices would be beneficial. For this reason nanopore manufacturing is pursued. Fabrication of pores of nanometer-sized diameter is a daunting task as their desired size is smaller then the resolution of standard industrial and available electron lithography techniques. The previously mentioned track-etch method can be used to create individual cone-shaped pores in \(\sim \mu\text{m}\) thin polymeric foils with tip apertures down to \(2 - 6\) nm [52]. However, their micrometer-long and moderately reproducible geometry and their device integration possibilities, are
Li produced the first solid-state nanopore in a thin silicon nitride membrane using an ion beam apparatus [53]. Initially a 60 nm diameter pore was created after which the increased ion beam exposure surprisingly decreased the pore dimension down to a 1.8 nm diameter. The size of the final nanopore was controlled using a feedback mechanism counting the number of transmitted ions. A similarly fabricated 5 nm diameter pore was successfully used to measure DNA induced current blockades. Storm developed solid-state nanopores in silicon oxide and used an electron beam to attain the desired dimensions [54]. First, a 20 nm diameter pore is fabricated in silicon using electron-beam lithography and chemical etching. After thermal oxidation the pore is subsequently exposed to a high-energy electron beam in a transmission electron microscope (TEM), shrinking the hole. Direct visualization of the process in the TEM results in an excellently controlled final nanopore diameter.

The successful use of solid-state nanopores in single-molecule translocation studies has resulted in a new and active area of research. The adjustable dimensions and surface characteristics of solid-state nanopores are expected to yield new possibilities for single-molecule sensing. The opportunities include separation, sizing and sorting of molecules [55]. Ultimately DNA sequencing, determination of the DNA code during single-molecule passage through the nanopore, might even be feasible. Nanopores can also contribute to scientific questions. The physics behind the translocation in naturally occurring biological pores, and the effects of nanometer-scale confinement on physical properties can be experimentally addressed. Solid-state nanopores thus show great promise as a versatile single-molecule tool for biotechnology and biophysics.

1.6 Outline of this thesis

This thesis reports on the fabrication and characterization of solid-state nanopores, and their use in single-molecule translocation studies. In the first part of this thesis we describe a new process developed for nanometer-sized pore fabrication. We investigate to what extent we can control their lateral shape and size, and explore the possibilities to influence their local surface composition and three-dimensional geometry.

The second part of this thesis discusses the ionic current noise measured on the fabricated solid-state nanopores. We describe the observed current fluctuations over the entire frequency range. Furthermore, we investigate alternative models describing the low-frequency noise characteristics.

The third and final part of this thesis shows DNA and protein-coated DNA
Chapter 1. Introduction

translocation through solid-state nanopores. We use different salt concentrations to study the effective charge of bare double-strand DNA molecules, and show the detection of fully protein-coated DNA molecules.

The outline of this thesis is as follows.

Chapter 2 reports on a novel fabrication process of solid-state nanopores. We use a high-intensity electron beam on thin free-standing membranes to create nanopores of controlled sizes, down to a 0.4 nm radius. We show that these nanopores can also serve as a template for the fabrication of nanoelectrodes, and we demonstrate their applicability in electrochemistry.

Chapter 3 describes the opportunities that arise from the use of an electron beam to create nanometer-sized pores. We identify a material-dependent response to the electron beam from differences in thickness profiles surrounding nanopores in pure SiN and SiO$_2$/SiN/SiO$_2$ membranes. We re-shape the initially fabricated nanopores in the SiO$_2$-containing membranes using electron beams of different intensities, and demonstrate their influence on the final geometry and the local surface composition.

Chapter 4 introduces the study of ionic current fluctuations in solid-state nanopores. We report on $1/f$ noise in the low-frequency regime, and large pore-to-pore variations in the noise power. We show that the low-frequency noise in nanopores with resistances values as expected scales linearly with the inverse number of charge carriers, in agreement with Hooge’s phenomenological relation. We describe the current noise in the high-frequency regime by a calculation of the Johnson noise. We use the obtained results to calculate signal-to-noise ratios for DNA translocation, yielding the salt concentration which maximizes the detection efficiency for a given nanopore diameter.

Chapter 5 develops an alternative model to Hooge’s relation for the low-frequency $1/f$ noise in solid-state nanopores. We give a theoretical description of the expected ionic current fluctuations in nanopores as originating from surface charge fluctuations. We compare the two models to the obtained low-frequency noise data and show that Hooge’s relation offers the best description over the entire salt range probed.

Chapter 6 studies the large pore-to-pore variations in the magnitude of the low-frequency noise. We show a correlation between a decreased nanopore conductance and increased amount of ionic current fluctuations. We report on anomalous ionic conductance profiles, measured using a moving laser focus. We
show that the presence of a nanometer-sized gaseous bubble inside a nanopore can explain the anomalous behavior, and act as a source of conductance and noise variability.

Chapter 7 reports on ion and DNA transport through solid-state nanopores at different salt concentrations. We show that the salt-dependent ionic conductance of nanopores deviates from bulk behavior, and we successfully model the conductance by taking the chemical reactivity of the nanopore surface into account. We demonstrate that the DNA translocation induced current changes in nanopores gradually change from negative to positive values, when lowering the salt concentration. We develop a model describing the data which incorporates the DNA area and its counter ions, and we infer a value of the effective electric charge of the DNA.

Chapter 8 studies translocation of RecA-coated DNA molecules through solid-state nanopores. We identify the formation of nucleoprotein filaments along the full length of the DNA molecules. We show that translocation experiments result in large conductance blockades with a wide variation in time duration. We compare the RecA-coated DNA translocation induced conductance blockades to measurements performed on bare DNA, and we deduce the diameter of the nucleoprotein filament. We present the event rate of RecA-coated DNA as a function of voltage, and show an exponential dependence at low voltages and a constant rate at high voltages.

References

Chapter 1. Introduction


Chapter 2

Fabrication and characterization of nanopore-based electrodes with radii down to 2 nm

We report on the fabrication and characterization of gold nanoelectrodes with carefully controlled nanometer dimensions in a matrix of insulating silicon nitride. A focused electron beam was employed to drill nanopores in a thin silicon nitride membrane. The size and shape of the nanopores were studied with high-resolution transmission electron microscopy and electron-energy-loss two-dimensional maps. The pores were subsequently filled with gold, yielding conically shaped nanoelectrodes. The nanoelectrodes were examined by atomic and electrostatic force microscopy. Their applicability in electrochemistry was demonstrated by steady-state cyclic voltammetry. Pores with a radius down to 0.4 nm and electrodes with radius down to 2 nm are demonstrated.

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2.1 Introduction

The ability to fabricate electrodes of accurately controlled nanometer dimensions is a key element in several areas of nanoscience, including molecular electronics and nanoelectrochemistry. Point contacts have been widely used in quantum transport studies [1, 2] and to probe electrical conduction through a limited number of molecules down to the single-molecule level [3–6]. In the context of electrochemistry, the main fundamental interest in these devices lies in their unique properties for the study of electron-transfer kinetics [7, 8] and mass-transport mechanisms in localized nanometer-scale volumes [9–11]. In general, as the size of an electrode is reduced, it is possible to obtain higher sensitivity, a lower detection limit, a smaller volume probed, and higher temporal resolution. Hence, metal nanoelectrodes are used in liquids in a broad spectrum of applications such as single-molecule detection [12], probing neurophysiological signals of small populations [13, 14], biomolecular sensors for medical diagnostics [15], and real-time monitoring of cell exocytosis [16]. The most common approach for electrode fabrication consists of electrochemically etching a thin wire down to a sharp conical shape followed by insulating the whole surface except for the very apex of the tip. The most broadly used insulating materials include electrophoretic paint [17, 18] and apiezon wax [8, 19]. Another preparation technique consists in stretching glass capillaries containing sealed microwires with a laser micropipet puller [20, 21]. Despite their large potential applicability, major obstacles still have to be overcome for the wider use of nanoelectrodes. In particular, the long-term instability and the difficulty in characterizing the size and shape of present-state nanoelectrodes lead to significant uncertainties in the analysis of experimental results. Here we report a novel method for the fabrication of metallic nanoelectrodes with carefully controlled dimensions of a few nm. We particularly focus on gold nanoelectrodes, although the extension of this process to other metals is straightforward. Our method consists of drilling a nanopore in a silicon nitride membrane using a focused electron beam, a method recently developed for nanofluidic applications [22–26]. We then fill the pore with an evaporated noble metal to yield conically shaped protruding electrodes. We present the characterization of these nanoelectrodes and demonstrate their applicability in electrochemical voltammetry.
2.2 Results

2.2.1 Fabrication of free-standing SiN membranes

Nanoelectrodes were fabricated by drilling nanometer scale pores in a thin SiN membrane and subsequently filling the nanopores with gold. A schematic representation of the desired electrode geometry is presented in Fig. 2.1, and the fabrication process is outlined in Fig. 2.2. The SiN membranes were manufactured following standard microfabrication technology from the semiconductor industry. Boron-doped Si (100) wafers with resistivities of 20 – 30 Ω cm were used. First, a three-layer structure was deposited by low pressure chemical vapor deposition (LPCVD) on both sides as follows: 20 nm of low-stress (silicon-rich) SiN, followed by 200 nm of SiO₂ and a 500-nm thick capping layer of low-stress SiN. The middle SiO₂ layer was deposited from tetraethoxysilane (TEOS). Square windows were then patterned in polymethylmethacrylate (PMMA) on the backside of the wafer with e-beam lithography and the pattern was transferred to the CVD layers with CHF₃/O₂ reactive ion etching (RIE) at 8 mbar (CHF₃ 50, O₂ 2.5 sccm, 50 W, etching time approximately 60 min). Using the SiN as a mask, the Si substrate was anisotropically etched in KOH (30 wt. %, 80 °C, etching time approximately 8 h), yielding free-standing 50x50 mm² membranes consisting of the three layer stack (Fig. 2.2(a)). In order to reveal the lowermost 20 nm SiN film, circular windows, 3 mm in diameter, were patterned in PMMA with e-beam lithography on the front side of the samples in the middle of the 50 mm membranes. Inside these regions the capping SiN and SiO₂ layers were removed with CHF₃/O₂ RIE and buffered HF etch respectively (Fig. 2.2(b)). Our membrane structure has two advantages over single-layer membranes. First, the capacitance of the membrane is greatly reduced as the 20 nm thickness has an area of only 7 mm² while the rest of the substrate is covered with an insulator thickness greater than 700 nm. Second, the mechanical strength is enhanced by the circular shape because the strain is homogeneously spread, in contrast to square-shaped membranes.

2.2.2 TEM-induced formation of a nanopore

Pores with radii between 0.4 and 100 nm were obtained in the thin membranes with a focused electron beam [23, 26] using a transmission electron microscope (TEM, Fig. 2.2(c)). Pores with radii in the range 1.5 - 4 nm were directly drilled with the electron beam. Here, an electron beam was tightly focused on the SiN membrane to an intensity of 10⁸ - 10⁹ e/nm² s. The high intensity of the electron beam breaks the covalent bonds and causes Si and N atoms to be sputtered away.
into the vacuum [26]. The time for pore formation can vary greatly depending on the beam parameters, ranging from less than 10 seconds for a 300 keV beam at approximately $10^9$ e/nm$^2$s to 5 minutes for a 200 keV beam at approximately $10^8$ e/nm$^2$s. In order to obtain bigger pores, a pore 2.5 - 4 nm in radius was first drilled and this pore was then expanded by broadening the electron beam (with an accompanying drop in intensity to values between $10^6$ and $10^7$ e/nm$^2$s). The pore growth was followed in real time by TEM imaging. When the pore achieved the desired size, the expanding process was stopped by substantially decreasing the beam intensity to less than $10^4$ e/nm$^2$s. Pores with radii smaller than 1.5 nm were obtained in a related manner. Here a 1.5 nm pore was drilled and a low-intensity ($10^5 - 10^6$ e/nm$^2$s) electron beam was then used to shrink this pore. Expansion and shrinkage are thought to be driven by surface-tension effects [23]. After pore formation, high-resolution transmission electron microscopy (HR-TEM) images were obtained in order to measure the pore diameter with high accuracy. This diameter determines the eventual electrode diameter.

### 2.2.3 Creating conically shaped nanoelectrodes

We have previously found [27] that sputtering Au to cover one side of a pore results in an inverted pyramid geometry with typical sidewall angles of 60. In order to obtain a convex electrode, a more sophisticated recipe was followed. A 0.5 nm Cr adhesion layer was evaporated at $10^{-7}$ Torr on the backside of the membrane followed by a 0.5 nm layer of Au without breaking the vacuum. The sample was then turned over and the front side of the pore was covered with SiO$_2$ by sputtering in an Ar plasma at 1 mTorr (power 100 W, rate 1 nm/min). A SiO$_2$ pit structure similar to those of Ref. 27 was obtained. This pit was subsequently filled with Au by a second backside evaporation (Fig. 2.2(d)). To guarantee coverage of the pore, both the SiO$_2$ and the Au layer thicknesses were
2.2 Results

Figure 2.2: Main steps of nanoelectrode fabrication process. (a) CVD three-layer deposition on Si, backside lithography and KOH etch. (b) Front-side lithography and etch of the SiN and SiO$_2$ layers forming a 20 nm SiN free-standing membrane. (c) Nanopore drilling in the SiN membrane with a focused electron beam. (d) Sacrificial SiO$_2$ layer sputtering and Au evaporation. After this step the sacrificial layer is removed in buffered HF.
at least 2.5 times the diameter of the pore. Finally the front SiO$_2$ was removed in buffered HF to reveal a structure as depicted in Fig. 2.1. All the materials employed in the fabrication are compatible with the use of organic solvents such as acetone or trichloroethylene (TCE). Therefore it is possible to chemically clean the electrodes if needed and to use them in non-aqueous solvents.

### 2.2.4 Nanopore shape characterized by electron-energy-loss spectroscopy

Figure 2.3(a) shows three different pores imaged with HR-TEM. Their radii are 12, 2.5 and 0.4 nm. The pore with a 2.5 nm radius was imaged right after formation by drilling with the focused beam. The 12-nm pore was first drilled and then slowly expanded in a controlled manner until this radius was achieved, whereas the pore with 0.4 nm radius was formed by shrinking a pore with initial radius of about 1.5 nm. The pores have a well-defined circular shape and their diameter, which is readily measurable with very high accuracy, can correspondingly be controlled to sub-nm precision. Note that the smallest pore has a diameter spanning only five atoms. In order to get some insight into the shape of the pores, we mapped their structure using electron-energy-loss spectroscopy (EELS). The local membrane thickness is determined by the log-ratio image of an energy-unfiltered and an energy-filtered image (with zero loss beam) [26, 28]. Because the EELS images acquired from a 20 nm thick membrane were quite noisy, it was not possible to obtain quantitative information from these data. We instead obtained thickness maps of pores drilled in thicker membranes. These were prepared by covering both sides of the SiN membranes with 20 nm sputtered SiO$_2$ and drilling the pores into this 60 nm membranes. Figure 2.3(b) shows the thickness variation around a pore 6 nm in diameter created in 15 minutes using an electron beam diameter of 8 nm (full width at half maximum). A three-dimensional reconstruction of a thickness map and a cross section across the pore are shown. The region over which the thickness of the thin film varies extends much further (radius about 80 nm) than the radius of the beam. This implies that rearrangement of material occurs in the 80 nm radius area even though this material is not strongly exposed to the electron beam. Since material is not observed to accumulate outside the 80 nm radius area, we believe that this material migrates to the central area, where it is sputtered away into the vacuum by the intense electron beam. From Fig. 2.3(b) and 2.3(c) it is clear that the material around the pore has a wedge shape and forms sidewall angles of approximately 55°. EELS characterization of different pores indicates that this slope can span a range 30° - 60° depending on the electron-beam intensity and the duration of the
2.2 Results

Figure 2.3: (a) HR-TEM images of nanopores with radii 12, 2.5 and 0.4 nm. (b) Thickness map (smoothed) of a 3-nm-radius pore obtained with EELS. (c) Cross section of the pore thickness profile (raw data) passing through the pore in (b). The absolute thickness was obtained by calibrating the relative thickness far from the pore to 60 nm.

drilling process. The radius of curvature at the constriction point is much smaller than the membrane thickness. We estimate an upper limit of 5 nm for the radius of curvature. While some differences may exist between this kind of membranes and 20 nm SiN membranes, we expect that a wedge shape of the nanopores is a universal property of electron-beam drilling. Similar results were obtained in 40 nm SiN membranes [26].

2.2.5 Characterization of the fabricated nanoelectrodes

The fabricated nanoelectrodes were characterized by atomic force microscopy (AFM) and simultaneously with electrostatic force microscopy (EFM). In the latter technique, each line of the image was first scanned in tapping mode, and then a second interleave scan was performed along the same line. During the interleave scan, the electrically grounded tip was raised 50 nm and it was constrained to follow the measured topography while a voltage of 2 V was applied to the substrate. Because the atomic forces are very short-ranged, the tip only cou-
Chapter 2. Nanopore-based electrodes with radii down to 2 nm

Figure 2.4: (a) AFM topography image of a nanoelectrode. (b) EFM image of the same electrode. (c) Cross section of the topographic image passing through the pore.

ples to long-range Coulomb interactions during the interleave scan. Electric field gradients are probed by recording the phase change of the cantilever oscillation. Because EFM is a direct probe of electric field, it is an ideal tool to locally distinguish conductive and insulating regions in the sample. An AFM topographic image showing a conical electrode is presented in Fig. 2.4(a). The corresponding EFM image shown in Fig. 2.4(b) confirms that the observed conical feature consists of metal surrounded by insulating material. The electrode presented in this figure has a radius (measured in TEM) of 24 nm. A topographic profile passing through the electrode is shown in Fig. 2.4(c). The wedge shape of the nanopores is apparent beside the nanoelectrode. Consistently with the expected geometrical shape, we measure a height of 30 nm from the SiN membrane to the tip of the electrode. The apparent radius of the electrode in AFM topography is 45 nm, but this is an upper limit only, because of the tip convolution. Unfortunately, the smallest electrodes could not be resolved with AFM due to the wedge shape of the nanopores (Fig. 2.3(c)) combined with tip convolution.

We demonstrate the performance of the fabricated nanoelectrodes by probing their voltammetric response to redox couples. Ferrocenylmethyltrimethylammonium (FcTMA$^+$) and ferrocenedimethanol (Fc(CH$_2$OH)$_2$) were employed as electroactive species and NH$_4$NO$_3$ was used as inert base electrolyte. Ferrocenylmethyltrimethylammonium hexafluorophosphate was synthesized by metathesis of the iodide salt (Lancaster, England) with ammonium hexafluorophosphate (Aldrich, USA) [27]. Ammonium nitrate (Merck, Germany) and Fc(CH$_2$OH)$_2$ (Aldrich, USA) were used as received. Aqueous solutions were prepared with
2.3 Discussion and conclusions

18 MW cm water from a Milli-Q purification system (Millipore, USA). Electrochemical measurements were performed in a 200 mL polydimethylsiloxane (PDMS) cell. The cell had two openings: a bottom one, 150 mm in diameter, that contacted the nanoelectrode and a macroscopic upper hole where a commercial Ag/AgCl electrode was placed as both reference- and counter electrode. The oxidation of the redox species in the presence of 0.5 M NH₄NO₃ was recorded in a two-electrode configuration using home-built electronics with a bandwidth of 3 Hz. At this salt concentration the Debye length is roughly 0.4 nm. Electroactive species concentrations in the solutions were determined from the voltammetric limiting current measured at 10 mm disc electrodes (BASi, USA).

Typical steady-state cyclic voltammograms are shown in Fig. 2.5. The observed sigmoidal shape of the current-voltage curves provides a signature of the good performance of the electrode surface. In addition to the transport-limited faradaic currents, a hysteretic offset is measured between the forward and backward scans, resulting from the parasitic dielectric response of the insulating membrane. This is especially clear for the smallest electrodes where the faradaic currents are in the sub-pA levels. It should be noted that this non-faradaic current is not the result of the metal-electrolyte double-layer capacitance. Instead, it is the effect of a dielectric relaxation of the membrane material [29, 30]. The transport-limited currents achieved for the neutral species Fc(CH₂OH)₂ are in good agreement with the expected diffusion-limited current [31]. For example, the bottom voltammogram shown in Fig. 2.5(a), displays a diffusion-limited plateau \( i_L = (5.2 \pm 0.2) \) pA. We can extract from this current an effective radius \( R_{eff} = (6.6 \pm 0.6) \) nm, in very good agreement with the measured radius \( R_0 = 6.5 \) nm. Here the \( R_{eff} \) is defined from the diffusion-limited current at a hemispherical electrode \( i_L = 2\pi F D C R_{eff} \), where \( F \), \( D \), and \( C \) are the Faraday constant, the diffusion coefficient and the bulk concentration, respectively. On the other hand, interesting deviations from the classical picture are observed in the case of the positively charge species FcTMA⁺. This anomalous transport behavior will be discussed elsewhere [11].

2.3 Discussion and conclusions

A strong advantage of this kind of electrodes is that their size can be independently characterized prior to their use without risking contamination of the metal surface. Our fabricated electrodes proved to be stable over long measuring periods. The voltammetric currents did not change over 36 hours of continuous measurements. A drawback is their inability to probe high temporal resolutions
Figure 2.5: Cyclic voltammograms obtained for different electrodes recorded in solutions of (a) Fc(CH$_2$OH)$_2$ and (b) FcTMA$^+$. In the last curve it is possible to see an enhancement of the noise in the regime where faradaic currents are recorded [11]. The voltammogram scan rates are: (a) 10 mV/s, (b, top) 0.5 mV/s, and (b, bottom) 0.1 mV/s.

due to the slow response of the insulating material surrounding them. This problem may be solved in the future by the use of a metallic guard layer set at the same potential as the electrode itself so that no potential drop is present across the dielectric membrane. Another simple way to avoid the slow dielectric response of the SiN membrane consists in working with thick polymer membranes such as those used in the fabrication of track-etched nanopores [24, 25, 32]. However, the slow response of the dielectric material presents no restriction in applications where the potential of the electrode is held constant, for example biomolecular sensing.

We have demonstrated a novel process for fabricating nanoelectrodes as small as 2 nm in radius. Pores as small as 0.4 nm in radius were achieved using electron beam drilling. The ultimate limiting factor in drilling small pores is the TEM resolution. We do not foresee any reason limiting the use of these pores to obtain sub-1 nm gold electrodes.

## 2.4 Acknowledgements

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References

Chapter 2. Nanopore-based electrodes with radii down to 2 nm


A highly focussed electron beam in a high-resolution transmission electron microscope (HRTEM) can be used to create nanometer-sized holes in thin membranes. Excellent control over their lateral shape and size (down to 0.8 nm in diameter) is obtained, and the nanopores can be directly visualized during the fabrication process. However, their three-dimensional geometry and local surface composition is generally not characterized nor controlled. Here, we measure profiles of the thickness and local material composition of nanopores, and how these change upon exposure to electron beams of different intensity. We identify a material-dependent response to the electron beam by using pure SiN as well as SiO$_2$/SiN/SiO$_2$ membranes. Thickness profiles around nanopores fabricated in SiN membranes show small ($\approx$ 40 nm radius) depletion areas with large sidewall angles ($75^\circ$), whereas those in SiO$_2$-containing membranes show large ($\approx$ 70 nm radius) depletion areas with small sidewall angles ($55^\circ$). Furthermore, we re-shape initially fabricated small nanopores in SiO$_2$-containing membranes by using either a de-focussed or a highly focussed electron beam, and show its influence on the sidewall angle and local material composition near the nanopore. These experimental results provide a guide for optimizing nanopores for single-molecule detection.
3.1 Introduction

Transmission electron microscopy (TEM) can not only give chemical and electronic-structure information of materials down to an atomic level, but it can also be utilized as a means to fabricate nanostructures with a high-intensity probe. Since the electron beam typically has a nanometer or sub-nanometer width, changes will occur in a nanometer range. Therefore the high-intensity electron beam can be a very attractive tool in nanotechnology, and several applications have already been reported. Examples include the fabrication of nanometer-sized Si dots and wires [1], the welding of metallic nanowires and carbon nanotubes [2, 3], the fabrication of subnanometer-sized Au wires [4], and the manufacturing of electrodes with nano-metersized gaps [5]. The high-intensity electron beam in a TEM can also be employed to create nanopores in thin free-standing membranes [6-8]. These solid-state nanopores are used to detect and characterize DNA and RNA with single-molecule resolution [9], comparable to translocation measurements performed on biological phospholipid-embedded protein channels [10]. Solid-state nanopores have significant advantages over protein channels, as they are functional in a wider range of temperatures, solvents, and voltages and offer possibilities for device integration. Since nanopores created by the high-intensity electron beam can be directly imaged in the TEM during fabrication, a high degree of control over their lateral shape and size is obtained. However, their three-dimensional geometry and local surface composition is generally not controlled and characterized, whereas these are important in order to model translocation measurements [11]. Furthermore, it has been suggested that local surface hydrophobicity and roughness surrounding nanopores can result in undesirable low-frequency noise characteristics [12]. Nanopore geometries determined in 40 nm SiO$_2$/SiN [7], and 50 nm Si$_3$N$_4$ membranes [8, 13] show a very different sidewall abruptness, demanding further investigation.

In this paper, we study the effect of different membrane materials and electron beam intensities on the shape and composition of nanopores. First, we combine electron energy loss spectroscopy (EELS) and high resolution transmission electron microscopy (HRTEM) to show that pure SiN and SiO$_2$/SiN/SiO$_2$ membranes have different sensitivities to the electron beam. In membranes containing SiO$_2$, small particles are generated which we identify as being Si-rich. Additionally, we find that nanopores fabricated in SiN membranes show small depletion areas with large sidewall angles, whereas nanopore fabricated in SiO$_2$-containing membranes show large depletion areas with small sidewall angles. Secondly, we investigate the possibility to re-shape nanopores created in SiO$_2$/SiN/SiO$_2$ membranes. We show that the initial nanopore geometry can be changed to create either smaller
or larger sidewall angles by using a de-focussed or highly focussed electron beam, respectively. Changes in the local material composition are studied using EELS and scanning transmission electron microscopy (STEM). We demonstrate the influence of different electron beams on the atomic oxygen-to-nitrogen ratio near the nanopore. We achieve a locally larger or smaller oxygen-to-nitrogen ratio using a de-focussed or highly focussed electron beam, respectively.

3.2 Materials and methods

Membranes are fabricated using standard semiconductor microfabrication processes. First, a three-layer structure was deposited on both sides of a $20 - 30 \, \Omega \text{cm}$, boron-doped, Si (100) wafer by low pressure chemical vapor deposition (LPCVD): 20 to 60 nm of low-stress (silicon-rich) silicon nitride (SiN), followed by 200 nm of SiO$_2$, and a 500 nm thick capping layer of low-stress SiN. Square windows are patterned in these layers on the backside of the Si wafer, by e-beam lithography and CHF$_3$ reactive ion etching. Using the SiN as a mask, the Si substrate is anisotropically etched in KOH solution (29 weight %) at 80$^\circ$C for approximately 8 hours. This results in free-standing $50 \times 50 \, \mu\text{m}^2$ membranes consisting of the three-layer stack. In the middle of these membranes, we subsequently open a 5 $\mu$m wide region to expose only the lowermost 20 to 60 nm SiN layer. We remove the capping SiN and SiO$_2$ layers by reactive ion etching and a wet HF etch, respectively. To fabricate the SiO$_2$/SiN/SiO$_2$ triple layer membranes we additionally deposit a 20 nm siliconoxide film on each side of a 20 nm thin SiN membrane by sputtering in Ar plasma.

Nanopores are fabricated and monitored in a FEI Tecnai (S)TEM operated at accelerating voltages of 200 kV and equipped with a field-emission gun, a scanning detector, and a high-resolution Gatan Imaging Filter (HRGIF) [14, 15]. An electron beam of 2 to 10 nm diameter (full width at half maximum) and a 2 to 7 nA beam current is used for initial nanopore fabrication. The oxygen/nitrogen ratio is determined by operating in STEM mode with a spot size of approximately 0.5 nm, a camera length of 30 to 50 mm, a HRGIF entrance aperture of 2 mm, and an energy dispersion of either 0.2 or 0.5 eV/pixel. The Energy-Filtered Transmission Electron Microscopy (EFTEM) images and thickness profiles are recorded using a 5 mm HRGIF entrance aperture and a 4 and 10 eV energy window, respectively.
3.3 Results

3.3.1 Nanopores fabricated in SiN and SiO$_2$/SiN/SiO$_2$ membranes

Figure 3.1(a) and (b) show images of two nanopores of 8 nm diameter, fabricated in 20 nm SiN and 60 nm thin SiO$_2$/SiN/SiO$_2$ membranes under similar drilling conditions. Whereas the nanopore itself looks identical (see insets), a relatively large ($\approx 100$ nm) region surrounding the nanopore is different. Small particles with a size of approximately 3 nm can clearly be seen in the membrane containing SiO$_2$ (Fig. 3.1(b)), whereas these particles are not present in the pure SiN membrane (Fig. 3.1(a)). To exclude a film thickness effect, we also tested 40 and 60 nm thin SiN membranes, and these specimens did also not show the formation of the $\approx 3$ nm sized particles (data not shown). We have further investigated the particles by EFTEM. Figure 3.1(c)-(e) shows EFTEM images of a 14 nm diameter nanopore in a SiO$_2$/SiN/SiO$_2$ membrane using different energy windows: an elastic image ($-2$ to $+2$ eV, Fig. 3.1(c)), and images around the Si (15 to 19 eV, Fig. 3.1(d)), and SiN and SiO$_2$ bulk plasmon peaks (21 to 25 eV, Fig. 3.1(e)). The particles are clearly visible in the 15 to 19 eV EFTEM image of Fig. 3.1(d), identifying them as Si-rich. No lattice fringes are found in the high resolution electron microscopy (HREM) images of these particles. More detailed information on the valence state of the Si in these particles can not be given since their contribution can not be separated due to their intrinsic energy width. The presence of the Si-rich particles in membranes containing SiO$_2$ is not related to an intermediate oxidation stage [16], but results from exposure to the electron beam. The formation of crystalline [17], and amorphous Si-features in SiO$_2$ [1] by the use of a high-intensity electron probe was reported previously. We conclude that the pure SiN and SiO$_2$ containing membranes respond differently to the electron beam, resulting in the formation of $\approx 3$ nm sized Si particles for the latter. Moreover, the high-intensity electron beam influences the SiO$_2$-containing membranes over a large region surrounding the nanopore, of approximately 100 nm in diameter.

In addition, we investigate the thickness-variation profiles surrounding nanopores in SiN and SiO$_2$-containing membranes. Figure 3.2 shows the thickness variation around 9 ± 1 nm diameter nanopores in 60 nm thin pure SiN (grey) and SiO$_2$/SiN/SiO$_2$ membranes (black). The nanopores are symmetrically located around the 0 nm position, and the membranes can be seen to be approximately 60 nm thin at large distances from the nanopores. The thickness profiles surrounding both nanopores show large differences. The nanopore in the
Figure 3.1: TEM images of two 8 nm diameter nanopores fabricated in (a) a 20 nm thin SiN and (b) a 60 nm thin SiO$_2$/SiN/SiO$_2$ membrane under similar drilling conditions. HRTEM images of the nanopores are presented in the inset. Whereas the nanopores themselves look identical, the membrane surrounding the nanopore shows the either the absence (a) of presence (b) of small-sized particles. EFTEM images of a 14 nm diameter nanopore in a SiO$_2$/SiN/SiO$_2$ membrane acquired with different energy windows (a) $-2$ to $+2$ eV, (b) 15 to 19 eV and (c) 21 to 25 eV. The small-sized particles are identified as Si-rich as they are most clearly visible in image (d).

SiN membrane is rather sharply defined with a $\approx 40$ nm radius depletion area ($< 90\%$ original thickness) and a 75$^\circ$ sidewall angle. In contrast, the nanopore profile in the SiO$_2$-containing membrane is wedge-shaped with a $\approx 70$ nm radius depletion area and a 55$^\circ$ sidewall angle. Both nanopores are fabricated using similar electron beam conditions. We compare the measured thickness-variation profiles of Fig. 3.2 to the sizes of the electron beams used. The nanopores are fabricated using two different electron beams: one being 9 nm wide (full width at half maximum), and a second being $\approx 4$ to 6 times larger. The small electron beam is used to create the nanopores, whereas the larger beam is used to inspect nanopore formation. The latter is only used for approximately 3 percent of the total exposure time. Assuming a Gaussian beam profile and a linear material loss with beam dose, the material depletion profile for the experimental combination of the two electron beams can be calculated. It is shown by the dashed
line in Fig. 3.2. The calculated depletion profile is remarkably small compared to the extent of the wedge-shaped area of the nanopore in the SiO$_2$-containing membrane. Evidently, the measured nanopore profile is not directly related to the size of the two electron beams used. Similarly, much smaller electron beam profiles are observed when a very small electron beam (0.1 – 0.15 nm) is used to fabricate nanopores in 40 nm thin SiN membranes, as shown in the supplementary information. These findings indicate that lateral diffusion processes play a role during the formation of nanopores. The results presented show that the different thickness profiles of nanopores in SiN and SiO$_2$/SiN/SiO$_2$ membranes, result from differences in material response to the electron beam.

### 3.3.2 Electron beam sculpting of nanopores fabricated in SiO$_2$/SiN/SiO$_2$ membranes

A fabricated nanopore can be further exposed to the electron beam to alter its lateral size, shape, and possibly its three-dimensional geometry and local surface composition. Here we investigate the effect of electron-beam sculpting on the thickness profile and oxygen/nitrogen ratio around nanopores fabricated in 60 nm thin SiO$_2$/SiN/SiO$_2$ membranes. We use either a de-focused or a highly focused electron beam to enlarge initially fabricated nanopores. The de-focused beam equally irradiates the rim of the nanopore, whereas the focused electron beam is positioned in the center of the nanopore or scans over its edge.

Figure 3.3(a) and (c) show the initial thickness profiles of two 8 nm diameter
3.3 Results

Figure 3.3: Thickness variation (acquired by EFTEM) and subtraction profiles around two initially 8 nm diameter nanopores before (black) and after (grey) their enlargement by the use of (a,b) a de-focused or (c,d) a highly-focused electron beam. The wedge-shaped nanopore thickness profile is affected over its entire range by use of the de-focused beam, whereas the focused beam only changes the profile in close proximity to the nanopore. The intensity of the electron beam used is schematically indicated.

nanopores in black. Both profiles are again wedge-shaped with a large depletion area extending up to about 75 nm from the center of the nanopores. We enlarge these nanopores to 18 nm in diameter using the two different electron beams. Figure 3.3(a) and (c) respectively show the thickness profiles in grey after exposure to the de-focused (≈ 20 nm wide) or focused (≈ 10 nm wide) electron beam. The black and grey profiles shown in Fig. 3.3(a) can be seen to differ over the entire wedge-shaped depletion area. In contrast, the profiles of Fig. 3.3(c) differ only near the nanopore. This is also evident from Fig. 3.3(b) and (d) were we show subtraction profiles comparing the initial and enlarged nanopores. Material loss occurs over the total wedge-shape when using the de-focused beam (Fig. 3.3(b)), or only near the edge of the nanopore when using the focused beam (Fig. 3.3(d)). These results suggest that the initial nanopore geometry can be changed, depending on the intensity of the electron beam, to create nanopores with either shallow or steep sidewall angles. A demonstration of the latter is shown in the supplementary information.

Apart from changes in the three-dimensional geometry of nanopores, we also study electron-beam-induced changes in the local material composition. We combine EELS and STEM to examine the local oxygen and nitrogen content of initially fabricated and enlarged nanopores. Figure 3.4(a) and (b) show HRTEM images of the initial and enlarged nanopore, respectively. The black and grey dashed lines in the figures indicate the line scan of the STEM probe. We again
enlarge nanopores using the two different electron beams. Figure 3.4(c) shows atomic distribution profiles of the initial (black) and final (grey) nanopore, enlarged by exposure to a de-focused beam. Profiles of a nanopore enlarged by a highly focused electron beam are presented in Fig. 3.4(e). The center of the nanopore is again located at 0 nm. The oxygen core-loss signal is larger than the nitrogen signal, mainly due to a higher atomic presence of oxygen in the three-layer membrane. All profiles show large depletion areas surrounding the nanopore. The average radii of the oxygen and nitrogen depletion areas are approximately 30 and 75 nm, respectively. When comparing the initial (black) and enlarged (grey) nanopores it is again evident that the de-focused beam changes the entire depletion profile (Fig. 3.4(c)), whereas the focused electron beam only modifies the profile in close proximity of the nanopore (Fig. 3.4(e)). We use the measured profiles of Fig. 3.4(c) and (e) to calculate the local atomic oxygen-to-nitrogen ratio. Figure 3.4(d) and (f) present these ratio profiles of the initial (black) and enlarged (grey) nanopores using either the de-focused or the highly focused beam, respectively. Both profiles show large noise near the nanopore opening, due to the small signal obtained on the locally very thin membrane, and comparable oxygen-to-nitrogen atomic ratios of $\approx 3.8$ at large distances ($> 150$ nm) away from the nanopore. The measured ratio agrees well with a value of 4, expected for membranes containing 40 nm SiO$_2$ and 20 nm SiN. At an approximately 20 to 80 nm distance from the nanopore center, we measure smaller oxygen to nitrogen ratios ($< 3.8$). Apparently, the top and bottom SiO$_2$ layers are more readily removed by the electron beam compared to the intermediate SiN layer. The lower oxygen-to-nitrogen ratio most likely results from a combination of differences in material response to the electron beam and material removal at the entrance and exit plane during nanopore fabrication [7]. From the profiles of the initial and enlarged nanopore shown in Fig. 3.4(d), we conclude that the use of a de-focused electron beam to enlarge nanopores results in a lower oxygen-to-nitrogen ratio over the whole nanopore depletion profile. In contrast, the use of a highly-focused electron beam to enlarge nanopores does not significantly alter the local oxygen-to-nitrogen ratio, as can be seen in Fig. 3.4(f). As a result, the local atomic oxygen-to-nitrogen ratio near a fabricated nanopore can be changed using different electron beam configurations. We can also inspect the formation of Si-rich particles in these membranes. Upon comparing the HREM images (i.e. Fig. 3.4(b)) with the oxygen distribution profile, we conclude that the Si-rich particles are present in areas where $\gtrsim 20\%$ of the oxygen is lost.
3.4 Discussion and conclusion

We identify a material-dependent response to the electron beam of pure SiN and SiO$_2$/SiN/SiO$_2$ membranes, resulting in different thickness profiles surrounding nanopores. Nanopores fabricated in SiN versus SiO$_2$-containing membranes show much smaller depletion areas ($\approx$ 40 versus $\approx$ 70 nm radius) and larger sidewall angles ($75^\circ$ versus $55^\circ$). Their three-dimensional geometry not deducible from these measurements, and as an example we show two different possibilities in in the inset of Fig. 3.2. Kim et al. [8, 13] previously reported the three-dimensional geometry of nanopores fabricated in 50 nm thin Si$_3$N$_4$ membranes, showing nanopores with a truncated double-cone structure. Upon, assuming an identical geometry we can also deduce the three-dimensional sidewall angle $\beta$, using $\beta = 2\arctan(tan(\alpha)/2)$ (see the inset of Fig. 3.2). The thickness profile surrounding the nanopores in SiN membranes gives $\beta = 124^\circ$ in good comparison to the value of $132.7 \pm 12.4^\circ$ reported by Kim et al [13]. The extended thickness profiles measured for nanopores fabricated in SiO$_2$/SiN/SiO$_2$ membranes are similar to the previously reported profiles in SiN/SiO$_2$ double-layer membranes [7]. The three-dimensional geometries of our fabricated nanopores are currently under
The radii of the depleted areas in both membranes are found to be larger than the size of the electron beam used to fabricate the nanopores. This indicates that there must be substantial diffusion of material during nanopore fabrication. Possible driving forces for this diffusion may result from the energy dissipated from the central part of the electron beam, the minimization of the surface energy [6], the chemical potential related to the composition gradient, and the backscattering of primary, secondary, and Auger electrons. All these forces depend on the material composition of the membrane. As a result, one expects to find different nanopore geometries for different membrane materials.

The material-dependent response to the electron beam is furthermore given by the absence or presence of Si-particles in SiN and SiO$_2$/SiN/SiO$_2$ membranes, respectively (Fig. 3.1). Si-rich particles are found to form in SiO$_2$/SiN/SiO$_2$ membrane areas where $\approx 20\%$ of the oxygen is lost, whereas they are not observable in SiN membranes even when up to $80\%$ of the N is removed (data not shown). All these results suggest that the three-dimensional geometry of electron beam fabricated nanopores depends on the membrane material composition.

In addition, we have shown that the thickness and local surface composition profile of initially fabricated nanopores in SiO$_2$/SiN/SiO$_2$ membranes can be changed. The use of a de-focused electron beam does not significantly change the thickness profile, but does result in lower oxygen-to-nitrogen ratios near the nanopore. Apparently, the oxygen is more easily removed than the nitrogen under these conditions. In contrast, the highly focused electron beam is seen to only affect the initial nanopore locally, resulting in larger sidewall angles and no changes in the oxygen-to-nitrogen ratio of the area surrounding the nanopore. Control over the three-dimensional structure and local surface properties of nanopores is of importance to improve the reliability of these sensors [12, 18], and to interpret results of translocation measurements [11].

In conclusion, we have shown that pure SiN and SiO$_2$/SiN/SiO$_2$ membranes respond differently to the electron beam, resulting in different thickness profiles surrounding nanopores. We can change the geometry and the local surface composition of initially fabricated nanopores by using electron beams of different intensities. The demonstrated material dependence and the electron beam sculpting of nanopores might enable the fabrication of improved and well characterized nanopores.
3.5 Acknowledgements

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References


Supplementary Information

This appendix contains additional information supporting the main text.

3.A Nanopore formation using a very small probe

We fabricate nanopores in thin SiN membranes with a very small diameter electron beam. For these experiments we use a STEM located at IBM in Yorktown Heights, which is based on the VG Microscopes HB501 STEM with the addition of a quadrupole-octupole aberration corrector [1]. We use an electron beam with spot sizes of 0.1 to 0.15 nm and currents between 30 and 50 pA (at 120 kV) on a 40 nm thin pure SiN membrane. Figure S.1(a) shows the STEM image of the resulting \( \approx 4 \) nm diameter nanopore. The formation of the nanopore, i.e. the total removal of the SiN membrane, is confirmed by a contrast comparison between the vacuum and the nanopore. The SiN membrane can be seen to contain Pt particles, as it was first cut out of a membrane using a focused ion beam (FIB) and subsequently mounted on a Cu grid by Pt deposition on the membrane-grid interface. The nanopore is fabricated within 30 minutes, during which the electron beam slowly changed position over \( \approx 7.5 \) nm in one direction due to drift (\( \approx 0.25 \) nm/min). The anisotropic wedge shape of the nanopore represented in Fig. S.1(b) reflects the drift along the B-B’ line. Figure S.1(c) shows the line intensity scaled to membrane thickness along this line, resulting in steep nanopore sidewall angles of 85° on the B’ side, and 73° on the B side, as indicated in the figure. The obtained nanopore thickness profile of the 4 nm diameter nanopore in SiN shows a large depletion area compared to the electron beam beam size (as indicated in Fig. S.1(b)), and large sidewall angles. The relatively large depletion area most probably results from SiN diffusion.

3.B Electron-beam sculpting of nanopores with steep sidewall angles

We demonstrate the ability to attain steep sidewall angles by electron beam sculpting of nanopores in 60 nm thin SiO\(_2\)/SiN/SiO\(_2\) membranes. A nanopore with an initial 57° sidewall angle is enlarged to \( \approx 80 \) nm in diameter using a highly focused electron beam which is scanned along the edge of the nanopore. Figure S.2(a) gives the measured thickness profile of the enlarged nanopore. The
Figure S.1: (a) STEM image of a 4 nm diameter nanopore formed with a 1 – 1.5 Å electron beam. The bright spots represent Pt particles, used to mount the 40 nm SiN membrane. The directions of the line scans are indicated. (b) Intensity along the lines AA’ and BB’. The BB’ profile is more shallow due to electron beam drift. The black line shows the profile of the high-intensity electron beam. The affected area surrounding the nanopore is large compared to the size of the beam. (c) Thickness profile of the nanopore along the BB’ line, obtained by scaling the line intensity to the membrane thickness. The profile has steep sidewall angles, as indicated.

depletion area of this re-shaped nanopore is clearly much smaller (≈ 40 nm from the edge) as compared to initially fabricated nanopores in SiO$_2$/SiN/SiO$_2$ membranes (cf. Fig. 3.2, Fig. 3.3(a) and (c)). Moreover, the sidewall angle is drastically increased to 83°, resulting in a near perfectly steep profile. Figure S.2(b) shows the atomic oxygen and nitrogen profiles of the nanopore. Both profiles display relatively small depletion areas (as compared to initially fabricated nanopores) and large sidewall angles, as expected from the thickness profile of Fig. S.2(a). No Si-rich particles are identified near the resulting nanopore.

Figure S.2: (a) Thickness variation (acquired by EFTEM) around a 90 nm diameter nanopore. The nanopore is sculpted using a highly-focused electron beam. The depletion area is about 40 nm wide, with steep sidewall angles of 83°, as indicated. (b) The atomic O (black) and N (grey) distribution profiles, showing identical steep edges. The inset in (b) shows a HRTEM image of the nanopore and a 100 nm scale bar.
References

Chapter 4

Noise in solid-state nanopores

We study ionic current fluctuations in solid-state nanopores over a wide frequency range and present a complete description of the noise characteristics. At low frequencies ($f \lesssim 100$ Hz) we observe $1/f$-type of noise. We analyze this low-frequency noise at different salt concentrations and find that the noise power remarkably scales linearly with the inverse number of charge carriers, in agreement with Hooge’s relation. We find a Hooge parameter $\alpha = (1.1 \pm 0.1) \times 10^{-4}$. In the high-frequency regime ($f \gtrsim 1$ kHz), we can model the increase in current power spectral density with frequency through a calculation of the Johnson noise. Finally, we use these results to compute the signal-to-noise ratio for DNA translocation for different salt concentrations and nanopore diameters, yielding the parameters for optimal detection efficiency.

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4.1 Introduction

Nanometer-sized pores can be used as versatile sensors for single biomolecules such as DNA, RNA, or proteins. The charged molecules are electrophoretically driven through the nanopore, resulting in temporal changes of the ionic current. The technique was first demonstrated by measuring the passage of DNA and RNA through the protein pore α-hemolysin [1]. More recently, solid-state nanopores were developed and used to measure the traversal of polynucleotides [2]. These translocation experiments have already addressed a wide range of interesting properties of nucleic acids [3]. Fabricated solid-state nanopores have obvious advantages over their biological counterparts, such as high stability, adjustable geometry and surface properties, and the potential of integration into devices. However, to date, they have been accompanied by a large variability in low-frequency noise, which limits their sensitivity and reliability [4, 5]. Studies of the ionic current noise can provide detailed information on dynamic processes occurring in the nanoscale volume of a single nanopore, and can help to improve and optimize nanopore characteristics. In protein pores, the protonation of ionization sites [6], the transport of sugars [7–10], ATP [11] and antibiotic molecules [12], and the conformational dynamics of protein pores [13] were all detected by studying ionic current fluctuations. On fabricated nanopores, only a few noise studies were performed so far, which related an increased low-frequency noise to the motion of polymeric subunits constituting the channel walls [14], and to the presence of nanometer-sized bubbles (nanobubbles) inside the nanopore [15].

In this article, we present a complete picture of the current noise of fabricated solid-state nanopores by addressing both the low- and the high-frequency regimes. We first give a brief overview of the general characteristics of our nanopores, showing a linear current-voltage (I-V) relation with resistance values that can vary significantly from pore-to-pore. We compare current-time traces and power spectra of illustrative nanopores of similar diameter but substantially different resistance, and we find that, whereas the high-frequency noise is of comparable magnitude, the low-frequency $1/f$ noise can be dramatically different. We show that the high-frequency current power spectral density is well described by the Johnson noise in our electrical circuit. Subsequently, we study the low-frequency $1/f$ noise in nanopores with resistance values that fit the nanopore geometry, and we identify that this noise can be related to the number of charge carriers, as described by the Hooge relation. We conclude by using our results in a calculation of the signal-to-noise ratio of DNA translocation through these solid-state nanopores. Surprisingly, we find that large nanopores ($d_{\text{pore}} > 20 \text{ nm}$) have improved signal-to-noise ratios in low-salt compared to high-salt regimes.
4.2 Materials and methods

Single solid-state nanopores are fabricated in thin 20 nm low-stress SiN membranes. Most membranes were covered by 10–20 nm sputtered SiO$_2$ on each side. Formation of a nanopore results from the exposure of the membrane to a tightly focused electron beam using a transmission electron microscope (TEM) [16]. The resulting nanopores can be imaged directly in the TEM, as shown in Fig. 4.1(a) Inset. Details of the fabrication process are described elsewhere [17]. In our experiments, nanopores with diameters between 3.7 and 51.0 nm are used. Before use, the nanopores are either exposed to an oxygen flow at elevated temperatures (300, 600 or 900°C), flushed with ethanol, or subjected to an oxygen plasma. The pretreatment removes organic contaminants and enhances the hydrophilicity of the surface. The different pretreatments did not yield systematic differences for the nanopore resistance or noise. Nanopores were mounted in the setup using a microfluidic flow cell. Solutions are prepared by adding Milli-Q filtered water (Millipore) to a stock solution of 1 M KCl with 10 mM Tris-HCl buffer at pH = 7.5. Ionic currents are detected by Ag/AgCl electrodes, connected to an amplifier operating in resistive feedback mode (Axopatch 200B, Axon Instruments). The currents are low-pass filtered using an external 8 pole Bessel filter, with a cutoff frequency of 49.9 kHz. The signal is digitized at 250 or 500 kHz. Power spectra result from ≈ 2 s of current recordings using Labview (National Instruments). The data are smoothed by averaging over an increasing number of nearest neighbor points. All calculations presented are performed using a cylindrical nanopore, with a length of $L_{\text{pore}} = 25$ nm, and surface charge values from ref. 15. The values of the amplifier configuration are given by $R_f = 500$ MΩ, $C_f = 1$ pF, $i_a = 3.2 \times 10^{-30}$ A$^2$/Hz and $e_a = 9 \times 10^{-18}$ V$^2$/Hz. For the calculation of the current power spectral density of our nanopore sample, we use $e_a = 6 \times 10^{-16}$ V$^2$/Hz, a value that is relatively high compared to the expected performance of the amplifier. This is likely to be the result of an overestimation of the value of $R_c$. The SNR is calculated by using an applied voltage of 100 mV, and $C_p = 30$ pF with $D = 2.7 \times 10^{-1}$. Integration of the current power spectrum is performed from 0.5 Hz.

4.3 Results

4.3.1 General nanopore characteristics

Figure 4.1(a) shows an example of $I-V$ measurements at 1 M KCl of six individual nanopores with different diameters (Fig. 4.1(a) Inset shows a transmission
electron micrograph of a 15.6 nm diameter pore). In all experiments, the $I - V$ curves display a linear relationship. Linear fits to the data yield the value of the nanopore resistance, and the resistance of 28 individual nanopores as a function of diameter is plotted in Fig. 4.1(b). As the nanopore diameter is increased from 3.7 to 51.0 nm, the resistance decreases from 480 to 3.5 MΩ. The measured resistance values show significant pore-to-pore variations, which we interpret as increases of the nanopore resistance from the resistance values expected by geometry. The solid line shows the expected resistance of a 25 nm long cylinder that scales as $1/d_{pore}^2$, where $d_{pore}$ is the nanopore diameter [15]. Although it coincides with some data points, many nanopores also exhibit a higher resistance. Nanopores with a large resistance value compared to the resistance of the cylinder ($\geq 2.5 \times$) are shown in grey.

Figure 4.1(c) shows current traces and histograms of two representative nanopores of similar ($\approx 21$ nm) diameter, which differ substantially in resistance. Their resistance values can be deduced from the differing currents as 9.1 and 34.7 MΩ, where the higher resistance value is large compared to the expected resistance of 4.7 MΩ for a 25 nm long cylinder. Both traces are recorded at an applied voltage of 100 mV and low-pass filtered at 10, and 1 kHz, as indicated. The 10 kHz filtered current traces (black and grey data in Fig. 4.1(c)) show strong differences in current noise, which is reflected in the width of their current histograms. The standard deviation of the current is 22.6 and 78.6 pA, for the black and grey traces, respectively. These differences become even more evident for the traces filtered at 1 kHz (see Fig. 4.1(c)). From a comparison of the top current traces, it is apparent that the current noise is greatly reduced upon additional filtering. However, the 1 kHz filtered bottom trace displays hardly any reduction of the large current fluctuations and width of the current histogram compared to the 10 kHz filtered grey trace. Indeed, upon filtering, the standard deviation of the current at 1 kHz decreases to 3.6 and 70.6 pA for the top and bottom traces, respectively. We conclude that the current fluctuations of the nanopore with the large resistance (bottom traces) can be attributed to excessive low-frequency noise, as is also evident from the current power spectral densities shown in Fig. 4.1(d). At frequencies below 200 Hz, both traces display $1/f$-type of noise, which differs by nearly 3 orders of magnitude. The black trace displays $1/f$ noise up to $\approx 200$ Hz, followed by an increase in current power spectral density up to $\approx 15$ kHz, and ending with a relative flat region before filter attenuation. For the grey trace, $1/f$ noise dominates the current power spectral density up to frequencies of $\approx 2$ kHz. At higher frequencies, the power spectrum appears flat until its attenuation by the low-pass frequency filter. Notice that, although the measured low-frequency noise is very different, the high-frequency
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Figure 4.1: General nanopore characteristics at 1 M salt. (a) Current-voltage characteristics of six individual nanopores with nanopore diameters as indicated. All curves show a linear $I - V$ dependence. (Inset) A transmission electron microscopy image of the 15.6 nm diameter nanopore. (b) Resistance values of 28 individual nanopores as a function of nanopore diameter. The solid line represents the resistance of a 25 nm long cylinder. Resistance values larger then 2.5 times the resistance indicated by the solid line are shown in grey. (c) Current recordings and histograms of two nanopores (at 100 mV) with substantially different resistance values, illustrating clear differences in current noise. The nanopores diameters are 20.8 nm (bottom traces) and 22.0 nm (top traces). The current was filtered at 10 and 1 kHz, as indicated. The top black and bottom histograms, shown on the right, are magnified along the x-axis to be visible on the same scale. (d) Current power spectral densities of the two nanopores used in (c), showing $1/f$ low-frequency noise of different magnitude and comparable high-frequency noise.

noise of the nanopores in Fig. 4.1(d) is quite comparable.

The differences in low-frequency $1/f$ noise dramatically affect the current-time characteristics of these nanopores. In general, the magnitude of the $1/f$ noise shows strong pore-to-pore variations, with excessive low-frequency noise for nanopores with relatively high resistance values (grey data points in Fig. 4.1(b)). Here, we study the low-frequency noise for nanopores with resistances close to the values as expected from the nanopore geometry. For completeness, the low-frequency noise data of all nanopores is presented in the supplementary information and Figs. S.1-S.5.
Chapter 4. Noise in solid-state nanopores

4.3.2 Modeling the high-frequency current noise

We model the high-frequency current power spectral density in our nanopore setup by a calculation of the Johnson noise [18]. For details on the calculations and fits performed in this section, see supplementary information. Taking the amplifier configuration into account, the current power spectral density can be calculated from the nanopore admittance $Y_s$ [19]. The nanopore is equivalent to a resistor $R_p$, in parallel with a capacitor $C_p$ resulting from the liquid contact to the silicon chip containing the nanopore. We account for the nonideal behavior of this capacitor by inclusion of an admittance $Y = \omega C_p D$ in parallel with $C_p$, with $D$ denoting the dielectric loss constant. The circuit is in series with a resistor $R_c$ that represents the (relatively small) resistance from the electrodes to the nanopore. To determine $Y_s$, we apply a voltage step to our nanopore sample and measure the current response. Figure 4.2(a) shows the current of a 15.6 nm diameter nanopore before ($t < 0$) and after ($t \geq 0$) a 4 mV voltage step. We first assume $D = 0$ and extract the values of $C_p$ and $R_c$ by fitting the expected current response to the data, as shown by the light grey line in Fig. 4.2(a). We obtain $C_p = 368 \pm 7$ pF and $R_c = 54.4 \pm 0.1$ kΩ. These values correspond to independently determined values of $C_p$ on membranes that do not contain nanopores, and values of $R_c$ measured without a nanopore sample present. The resistance $R_p$ is obtained from a dc current-voltage measurement, such as shown in Fig. 4.1(a), yielding $R_p = 67 \pm 2$ MΩ for the nanopore used. The value of the dielectric loss constant can now be determined from a closer inspection of the current response at long time scales. Figure 4.2(b) shows the current response to the applied voltage step from $t = 0$ to $t = 10^4 \mu s$ on a logarithmic scale. After an initial decrease, the current does not attain a steady-state value but continues to decrease up to $10^4 \mu s$ (equivalent to $500R_cC_p$). A fit of an ideal capacitor's response to the data is shown by the light grey line, which clearly fails to give a valid description for $t > 50 \mu s$. Alternatively, the current response at long time scales ($t > 40R_cC_p$) can be well described by Curie's law, which allows for a determination of the dielectric loss constant $D$ [20]. The dark grey line of Fig. 4.2(b) shows the best fit to the data for $t > 40R_cC_p$. We obtain $D = 0.27 \pm 0.07$, strongly deviating from $D = 0$ of an ideal capacitor [21].

Having determined the values of all components of the nanopore admittance $Y_s$, we can now calculate the current power spectral density and compare it to the measured values. Figure 4.2(c) shows the spectral density of the 15.6 nm diameter nanopore in black. The calculated power spectral density is shown by the solid line. For comparison, the effect of the filter used is taken into account. The modeled current power spectral density is flat at low frequencies,
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Figure 4.2: Modeling the high-frequency current noise in nanopores. (a) The applied voltage and the resulting current response as a function of time for a 15.6 nm diameter nanopore. The light grey line shows the best fit to the data. (b) The current response of (a) plotted on logarithmic scales for times up to $10^4 \mu$s. The light grey line is identical to the one plotted in (a), whereas the dark grey line is a fit to Curie’s law for $t > 40R_cC_p$. (c) Current power spectral density of a 15.6 nm diameter nanopore (black) and the calculated power spectrum (solid line). The dashed line results from an addition of the measured low-frequency noise to the calculated values.

corresponding to the Johnson noise of the resistor $R_p + R_c \approx R_p$, followed by an increase due to the capacitance $C_p$, and finally reaching again a constant value, given by the Johnson noise of the resistor $R_c$ alone. For high frequency values ($> 20$ kHz), the calculated spectrum decreases due to the filter cutoff. The model of the current power spectral density gives a good description of the data at frequencies $> 300$ Hz. For lower frequencies, the measured power spectrum cannot be modeled, because it is not possible to a priori calculate the $1/f$ noise. Addition of the measured low-frequency noise to the calculated power spectrum yields the dashed line of Fig. 4.2(c), resulting in an excellent description of the data over the whole frequency range.
4.3.3 Low-frequency $1/f$ noise in nanopores

We now turn to analyze the low-frequency $1/f$ noise in our solid-state nanopores. The nanopores analyzed have resistance values close to those expected from geometry. According to Hooge’s phenomenological relation for low-frequency $1/f$ noise, the noise power, $A$, should scale inversely with the number of charge carriers $N_c$ [22]:

$$\frac{S_I}{I^2} = \frac{A}{f} = \frac{\alpha}{N_c f},$$  \hspace{1cm} (4.1)

where $S_I$ is the current power spectral density, $I$ is the current, $f$ is the frequency, and $\alpha$ denotes the Hooge parameter, which quantifies the amount of low-frequency noise [23]. We tested this relation by measuring the low-frequency noise of individual nanopores at different salt concentrations, because this is a means of varying $N_c$.

Figure 4.3(a) shows the normalized current power spectral density $S_I/I^2$ at low frequencies of a single nanopore at five different salt concentrations. All traces show $1/f$-type of noise behavior, with variations up to 2 orders of magnitude. The data were fitted using $S_I/I^2 = A/f$, as shown by the solid lines in Fig. 4.3(a). As the salt concentration is increased, the value of the noise power decreases, with values of $A$ ranging from $6.4 \times 10^{-6}$ to $5.0 \times 10^{-8}$. Figure 4.3(b) shows the conductance and noise power of three individual nanopores measured at salt concentrations from 1 mM up to 1 M. We observe that the conductance strongly increases as the salt concentration is increased (black points in Fig. 4.3(b)), but that the noise power shows a strong decrease (grey points in Fig. 4.3(b)). The conductance does not show a linear dependence on potassium chloride concentration, due to the salt-dependent surface charge of the nanopore [15].

We now model the conductance in our nanopores to obtain a value for the number of charge carriers $N_c$ and subsequently use this value to validate the use of Eq. (4.1). The black line in Fig. 4.3(b) shows the conductance assuming a cylindrical nanopore geometry and the salt-dependent surface charge as given in ref. 15, which gives a good description of the data. From this we extract the number of ions in our nanopores $N_c$ at each salt concentration. The grey solid line now shows the best fit of the noise power $A = \alpha/N_c$ to the data, using Eq. (4.1) with one fitting parameter ($\alpha$) only. We find a value of $\alpha = (1.1 \pm 0.1) \times 10^{-4}$, close to typical values found for electronic devices [24]. The model describes the variation in noise power over the whole salt range remarkably well. Figure 4.3(c) and (d) show different representations of the same data. Figure 4.3(c) presents the noise power as a function of the calculated inverse
4.3 Results

number of charge carriers, showing the linear scaling (black line), in accordance with Hooge’s relation. Figure 4.3(d) shows the product of the noise power and the number of charge carriers, yielding a constant value of the Hooge parameter over the entire salt concentration probed. In conclusion, a variation of the noise power $A$ in terms of a variation in the number of charge carriers $N_c$, gives an excellent description of the data.

4.3.4 Signal-to-noise ratio for DNA translocation

Having fully characterized the current noise in the low- and high-frequency regime, we now turn to the signal-to-noise ratio ($SNR$) for DNA translocation. The $SNR$ is calculated as a function of salt concentration, yielding an optimal range for DNA translocation experiments. The $SNR$ is defined as

$$SNR = \frac{\Delta I}{I_{\text{noise,RMS}}},$$  \hspace{1cm} (4.2)

where $I_{\text{noise,RMS}}$ is the root-mean-square current noise, and $\Delta I$ is the absolute current change due to DNA translocation. Note that $I_{\text{noise,RMS}}$ equals the square root of the integral of the low- and high-frequency current power spectral densities, $I_{\text{noise,RMS}} = (\int_0^{BW} S_I df)^{1/2}$, where $BW$ is the bandwidth. Here we will consider low-frequency $1/f$-type of noise with $\alpha = (1.1 \pm 0.1) \times 10^{-4}$, as determined above. The current power spectral density $S_I$ at each salt concentration can then be calculated by using Eq. (4.1). The high-frequency current power spectral density follows from a calculation of the Johnson noise, with the value of $Y_s$ depending on the salt concentration. The DNA induced current change $\Delta I$ was experimentally measured before [15], and found to be linearly proportional to the salt concentration, with DNA translocation resulting in either decreases ($[KCl] > 0.4$ M) or increases ($[KCl] < 0.4$ M) of the ionic current. We assume the current change to be fully detectable at the used bandwidth of 10 kHz, which is valid for long ($\gtrsim 5$ kbp) DNA molecules (for $SNR$ calculations at 100 kHz, see supplementary information). Possible effects at very low salt concentrations, such as Debye layer overlap and changes in the number of condensed counter-ions have not been considered.

Figure 4.4 shows the resulting $SNR$ as a function of salt concentration for five different nanopore diameters. When the salt concentration is lowered, the $SNR$ initially decreases down to zero, because $\Delta I = 0$ when $[KCl] \approx 0.4$ M, and increases again reaching a local maximum at $[KCl] \approx 1 \times 10^{-3}$ M. At even lower salt concentrations the signal-to-noise ratio decreases again because the series resistance ($R_s$) becomes equal to or larger than the pore resistance ($R_p$), which results
Chapter 4. Noise in solid-state nanopores

Figure 4.3: Analysis of low-frequency $1/f$ noise in nanopores at different salt concentrations.

(a) The normalized current power spectral density of an individual nanopore at salt concentrations of 1 mM, 10 mM, 100 mM, 500 mM, and 1 M. The solid lines result from a fit of the data at each salt concentration to the formula shown. (b) Conductance and noise power of three nanopores from salt concentrations of 1 mM up to 1 M. Each individual nanopore has its own symbol. The black line shows the conductance of a cylindrical nanopore with an average nanopore diameter of $d_{pore} = 9.3$ nm, and a salt-dependent surface charge as given in ref. 15. The grey line shows the noise power in terms of the number of charge carriers using $\alpha = 1.1 \times 10^{-4}$. (c) Noise power as a function of the calculated inverse number of charge carriers of the data shown in (b). The black line indicates the linear scaling. (d) The value of the Hooge parameter over the salt concentration probed for the data shown in (b). The Hooge parameter results from the product of the noise power and the number of charge carriers. The black line shows the constant value $\alpha = 1.1 \times 10^{-4}$. 
4.4 Discussion and conclusions

We have investigated the ionic current noise in our fabricated solid-state nanopores. The high-frequency noise can successfully be modeled by a calculation of the Johnson noise using a simple electrical circuit representation. The capacitance, which stems from the liquid contact to the silicon chip containing the nanopore, and the relative small resistance from the electrodes to the nanopore determine the current noise in the high-frequency regime. In the low-frequency regime, we measure a $1/f$-type of noise behavior, which reflects the properties of our nanopores.

This noise power is found to vary from pore to pore, which results in dramatic differences in current-time characteristics. Nanopores with relatively high resistance values show excessive low-frequency $1/f$-noise. This agrees well with the previously proposed presence of nanobubbles, which can account for the observed

Figure 4.4: SNR calculated for DNA translocation through five nanopores with different diameters as a function of salt concentration. The nanopore diameters are indicated. The smaller the nanopore, the better the SNR. For large nanopores ($d_{\text{pore}} > 20\text{ nm}$) measurements performed at low salt concentrations yield the best SNR.

in a lower voltage drop over the nanopore. We have used $R_c = 200k\Omega/[KCl]$, but the same trend is observed for different values of $R_c$. Nanopores with smaller diameters have a better SNR for DNA translocation at all salt concentrations, as expected. At the commonly used potassium chloride concentration of 1 M, the SNR increases from 2 to 15 when the nanopore diameter is decreased from 100 to 3 nm. Higher values ($\approx 50$) are attainable at even higher salt concentrations. Surprisingly, we find that large nanopores ($d_{\text{pore}} > 20$ nm) have a better SNR in the low-salt compared to the high-salt regime. Detection of a single DNA molecule is even possible inside a nanopore with a diameter of $d_{\text{pore}} = 100$ nm, provided one measures at low salt concentrations.
low-frequency noise increase of partially blocked nanopores [5]. For nanopores with resistance values that correspond to the nanopore geometry, we show that the low-frequency $1/f$ noise behaves according to Hooge's phenomenological relation. These nanopores exhibit a linear scaling of the noise power with the inverse number of charge carriers, as is observed in many condensed-matter systems [24]. This behavior may also explain the unexpected scaling of the noise power with the inverse of the conductance found for biological pores [25]. For our solid-state nanopores, we obtain a Hooge parameter of $\alpha = (1.1 \pm 0.1) \times 10^{-4}$.

The results obtained offer a good description of the noise in our solid-state nanopores over the whole frequency range. They not only enable us to improve on characteristics of partially blocked nanopores by addressing their surface properties [4, 26], but also provide means to design and optimize nanopore experiments where the translocation of DNA is probed. As an illustration, we have shown that DNA translocation events are even detectable for nanopores with diameters as large as 100 nm, provided that measurements are performed at low salt concentrations ($\approx 1$ mM KCl).

### 4.5 Acknowledgments

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### References


The non-ideal capacitor, $C_p$, can also be described only in terms of the model constants $n$ and $h$ as obtained from the best fit to Curie’s law. The current power spectral density resulting from the use of these values is comparable to the calculated spectrum shown in Fig. 4.2(c).


Alternatively Hooge’s equation can be presented as $S_f/I^2 = \alpha/N_c f^\beta$. Using this equation yields $0.7 < \beta < 1.4$ and essentially the same results as presented.


Supplementary Information

This appendix contains additional information that was published as supporting information to the main text.

4.A Modeling the high frequency current noise

The current power spectral density, $S_I$, of an admittance $Y$ as determined by Johnson noise is given by

$$S_I = |Y|^2 S_V,$$  \hspace{1cm} (S.1)

where $S_V$ is the voltage power spectral density. For $S_V$ we can write

$$S_V = 4k_B T \text{Re} \left( \frac{1}{Y} \right),$$  \hspace{1cm} (S.2)

where $k_B T$ represents the thermal energy. The admittance of our nanopore sample follows from an electrical-circuit representation, which is shown in Fig. S.1(a). The parasitic capacitance is omitted here, as it does not affect our results for the frequency range under consideration. To calculate the current power spectral density measured in our setup, we also need to take the amplifier configuration into account, as shown in Figure S.1(b) [1]. The amplifier layout consists of an operational amplifier (grey triangle), with a feedback circuit of a resistor $R_f$ in parallel with a capacitor $C_f$. The operational amplifier generates noise with associated current and voltage power spectral densities of $i_a$ and $e_a$, respectively. The voltage power spectral densities of the nanopore sample $e_s$, and the feedback circuit $e_f$, follow directly from Eq. (S.2). We can now express the current power spectral density as

$$S_I = i_a + |Y_s|^2 (e_a + e_s) + |Y_f|^2 (e_a + e_f),$$  \hspace{1cm} (S.3)

where $Y_s$, and $Y_f$ denote the admittance of the nanopore sample and the feedback circuit, respectively. The current power spectral density can now be calculated from $Y_s$.

To determine $Y_s$, we apply a voltage step to our nanopore sample and measure the current response. We first determine the values of $C_p$ and $R_c$, by assuming $D = 0$. When the voltage is applied, it will initially drop entirely over $R_c$ ($\omega = \infty$), giving rise to the steep initial current increase. At long times, $t \to \infty$, the total voltage will drop over $R_c + R_p$ ($\omega \to 0$), resulting in lower current values.
**Supplementary Information**

**Figure S.1:** Electric circuit representations used to model the high-frequency current noise in nanopores. (a) Schematic of a nanopore sample. The non-ideal capacitance $C_p$ is represented by an ideal capacitor in parallel with a resistor, as indicated by the dotted square. All components are explained in the text. (b) Representation of the current amplifier with the nanopore sample attached. The figure shows the components of the feedback circuit and the voltage and current noise sources due to the amplifier $e_a, i_a$; the voltage noise source due to the feedback circuit $e_f$; and the voltage noise source due to the nanopore sample $e_s$.

Charging of the capacitor $C_p$ is present at intermediate times. Since $R_p \gg R_c$, the current response, $i(t)$, is given by

$$i(t) = \left( \frac{V_\Delta}{R_c} - I_\infty \right) \exp \left( \frac{-t}{R_c C_p} \right) + I_\infty,$$

where $V_\Delta$ is the applied voltage step, and $I_\infty$ the current at time $t = \infty$. The values of $C_p$ and $R_c$ can now be extracted by fitting Eq. (S.4) to the data [2].

The value of the dielectric loss constant, $D$, can be determined from a closer inspection of the current response at long time scales ($t > 40R_cC_p$). It can be well described by Curie’s law [3],

$$i(t) = \frac{V_\Delta}{ht^n},$$

where $h$ is a constant depending on the value of the capacitor and the type of dielectric, and $n$ is a constant depending on the dielectrical losses of the capacitor. The dielectric loss constant $D$ now follows from [4]

$$D = \tan \left( \frac{\pi(1 - n)}{2} \right).$$
Figure S.2: The normalized current power spectral density of six individual nanopores at 1 M salt, illustrating strong pore-to-pore variations. The voltages used are in between −200 and +200 mV. The solid lines result from a fit of the data of each nanopore to the formula shown.

4.B Low-frequency $1/f$ noise variation in solid-state nanopores

Figure S.2 shows the normalized current power spectral density $S_I/I^2$ at low frequencies for six different nanopores at 1 M salt. All nanopores show $1/f$-type of noise behavior, with however pore-to-pore variations up to 4 orders of magnitude. The data were fitted using

$$\frac{S_I}{I^2} = \frac{A}{f},$$  \hspace{1cm} (S.7)

where $I$ is the current, $A$ is the noise power, and $f$ is the frequency. The fits for each nanopore are shown by the solid lines in Fig. S.2. The values of $A$ obtained range from $3.6 \times 10^{-9}$ to $7.0 \times 10^{-5}$.

According to Hooge’s phenomenological relation for $1/f$ noise, the noise power should scale inversely with the number of charge carriers [5]. In Fig. S.3 we present the $1/f$ noise power as a function of the inverse number of charge carriers ($1/N_c$) for all nanopores used. We have indicated nanopores with resistances smaller and larger than 2.5 times the resistance of a 25 nm long cylinder with black and grey colors, respectively. The large resistance values exceed resistance values expected by geometry, and most likely result from the presence of a blocking object. The noise power of the nanopores shown in black varies from $A \approx 10^{-9} - 10^{-5}$, depending on the number of charge carriers. It scales linearly with the inverse number of charge carriers as shown by the black line in Fig. S.3. We have used the proportionality constant $\alpha = 1.1 \times 10^{-4}$. In contrast, the noise power of the partially blocked nanopores has a magnitude of $A \approx 10^{-5} - 10^{-4}$. In this case, the value of the low-frequency noise power is not only higher, but also independent of the number of charge carries. Whereas the low-frequency $1/f$ noise in nanopores
Figure S.3: The low-frequency 1/f noise power as a function of the calculated inverse number of charge carriers of twenty-seven individual nanopores. Nanopores with a resistance $< 2.5 \times 10^3$ and $\geq 2.5 \times$ the resistance of a 25 nm long cylinder are shown in black and grey, respectively. The black line shows a linear scaling using $\alpha = 1.1 \times 10^{-4}$.

with resistance values as expected from the nanopore geometry behaves according to Hooge’s relation, nanopores with relatively high resistance values show large 1/f noise and no scaling with $N_c$.

We now attempt to elucidate the mechanism responsible for the increased 1/f noise of the partially blocked nanopores. One might expect that the increased low-frequency noise can simply be explained by a reduced nanopore volume due to the partial blocking of the nanopores. A reduced number of charge carriers would then, according to Hooge’s relation, result in the increased 1/f noise. Consequently, the relative reduction in the number of charge carriers needs to match the relative increase in nanopore resistance. Figure S.4 shows the number of charge carriers expected compared to the number measured from the noise power $N_{c,geo}/N_{c,noise}$ as a function of the relative resistance of the nanopore $R/R_{cylinder}$ at 1 M salt. The number of charge carriers from the noise power was calculated using $N_{c,noise} = 1.1 \times 10^{-4}/A$. Whereas the relative resistance is increased by less than a factor of 10, the reduction in the number of charge carriers of the nanopores is approximately $10^2 - 10^3$. We therefore cannot conclude that the high noise of these nanopores is solely due to a reduced volume, since their relative resistance increase fails to account for the increased noise by several orders of magnitude. The excess low-frequency noise might instead be attributed to a dynamic particle present inside the partially blocked nanopores, giving rise to a strong increase of the low-frequency noise as a result of its dynamics. This agrees well with a previous proposal identifying nanobubbles, as the dominant low-frequency noise source [6]. In the case of biological and polymeric nanopores, 1/f noise has been attributed to the movement of subunits [7], or to the dynamics of the entire nanopore [8]. Noise generation by nanobubble nucleation, movement along the nanopore surface and/or dissolution is similar to these mechanisms.
Chapter 4. Noise in solid-state nanopores

4.C Signal-to-noise ratio for DNA translocation

Figure S.5 shows the SNR for DNA translocation through nanopores with three different diameters as a function of salt concentration. Here, the SNR is calculated up to frequencies of 10 kHz and 100 kHz as shown by the solid and dashed lines, respectively. The 100 kHz filtered SNR is evidently lower than the 10 kHz filtered calculations, for all nanopore diameters and salt concentrations. The signal-to-noise ratio is again better in the low-salt compared to the high-salt regime, the effect being even more pronounced for the SNR at 100 kHz. For a salt concentration of 1 M we find SNR values (at 100 kHz) ranging from 2.0 to 1.4, making measurements of DNA translocation impossible in practice. In contrast, the maximum attainable SNR values (at 100 kHz) are approximately 17, 13 and 9 at salt concentrations of 40 mM, 71 mM, and 89 mM for the 3 nm, 20 nm, and 50 nm diameter nanopores, respectively. Measurements performed at these lower salt concentrations do allow for the detection of a single DNA molecule with a 100 kHz bandwidth.

References


[2] For an ideal capacitance, $C_p$, the value of $R_p$ can also be extracted from a fit, since $I_\infty = V_\Delta/(R_c + R_p)$. However, due to the pronounced nonideal behavior of the capacitance $C_p$ and possible small voltage offsets, the value of $R_p$ extracted from a dc current-voltage measurement is less prone to error.

Figure S.5: Signal-to-noise ratio calculated for DNA translocation through three nanopores with different diameters as a function of salt concentration. The used sharp filter cutoff frequencies are 10 kHz and 100 kHz for the solid and dashed lines, respectively. The nanopore diameters are indicated in the figure.

Chapter 4. Noise in solid-state nanopores
Chapter 5

Comparison of two models for the low-frequency noise in solid-state nanopores

Low-frequency ionic current noise in solid-state nanopores poses a limitation to the time resolution achieved in translocation experiments. Recently, this $1/f$ noise was described to obey Hooge’s phenomenological relation, where the noise scales inversely with the number of charge carriers present. Here, we consider an alternative model in which the $1/f$ noise originates from surface-charge fluctuations. We compare the two models and show that, whereas the models are not easily discriminated in the low-salt regime, Hooge’s relation gives the best description for the low-frequency noise in solid-state nanopores over the entire salt regime from $10^{-3}$ to 1.6 M KCl.

Chapter 5. Comparison of two models for the low-frequency noise

5.1 Introduction

Single-molecules passing through electrolyte-filled nanopores cause temporal changes in the conductivity. This technique has been used to detect nucleic acids, and to study a wide range of their properties [1]. A variety of biological, synthetic and solid-state nanopores are used in these translocation experiments. The latter type is the obvious candidate for device integration and offers the best flexibility in nanopore size and experimental conditions. The temporal resolution for single-molecule translocation experiments is ultimately set by the level of ionic-current noise. Fast detection with low noise levels might eventually allow for the detection of local structures along nucleic acids such as single bound proteins, triple-strand structures, individual hairpins, and mismatched bases [1]. Noise studies will not only help to improve and optimize nanopore characteristics, but can also provide detailed information on dynamic processes occurring in the volume of a single pore, and contribute to the understanding of noise sources. The low-frequency ionic-current noise in solid-state nanopores, which poses a fundamental limitation to the detection, is of a $1/f$ spectral character [2–4]. This flicker noise in solid-state nanopores was recently found to obey Hooge’s phenomenological relation, where the noise power scales linear with the inverse number of charge carriers [4]. However, surface modifications have resulted in reduced $1/f$-noise levels in nanopores [2]. Although these results might be explained by improvements of the surface hydrophilicity [5], they raise the question to what extent surface-charge fluctuations can be responsible for the observed $1/f$ noise.

In this chapter, we consider low-frequency ionic-current fluctuations in fabricated solid-state nanopores. Next to the Hooge model mentioned above, we develop a model in which the $1/f$ noise originates from surface-charge fluctuations. Subsequently, we compare the predictions from these models to measurements of the $1/f$ noise power in nanopores as a function of salt concentration. We show that the surface-charge model and Hooge’s relation scale identical with salt at low salt concentrations, and as a result their validity cannot be discriminated in this regime. In the high-salt regime, however the surface-charge model fails to account for the observed low-frequency noise, in contrast to Hooge’s description. We thus verify that over the entire salt regime probed the noise is best described by Hooge’s relation.

Our analysis provides a tool to identify low-frequency noise originating from surface charge fluctuations and yields insight into different noise mechanisms.
5.2 Modeling of the low-frequency current noise

5.2.1 Surface charge fluctuations

Any thermally activated random process with a constant energy distribution generates a $1/f$ frequency spectrum [6]. We assume a range of activation energies for protonation and deprotonation of the oxide surface groups of the solid-state nanopores. Overall the charge of the surface is given by the dissociation of silanol groups, according to

$$\text{SiOH} \rightleftharpoons \text{SiO}^- + \text{H}^+. \quad (5.1)$$

Surface charge fluctuations are given in terms of fluctuations around the chemical equilibrium of Eq. (5.1). The number of charged sites per surface area is simply given by $\sigma/e$, where $\sigma$ represents the surface charge density and $e$ is the elementary charge. Given a total number of chargeable sites per area, $\Gamma$, we can express the amount of surface charge fluctuations, $\delta q$, and its power spectral density, $S_q$, as

$$\delta q = \left( \frac{\sigma (\Gamma - \frac{\sigma}{e})}{\Gamma} \right)^{1/2} \gamma F(t) \quad (5.2)$$

$$S_q = \left( \frac{\sigma (\Gamma - \frac{\sigma}{e})}{\Gamma} \right)^{\gamma^2} f \quad (5.3)$$

where $\gamma$ is a proportionality constant, $F(t)$ is a dimensionless noise function with a $1/f$ power spectrum, and $f$ is the frequency. The term in between brackets in Eq’s. (5.2) and (5.3) expresses the scaling between the surface charge fluctuations and the surface charge density. The fitting parameter $\gamma$ reflects the strength of fluctuations and has the dimension of charge.

Low-frequency $1/f$ noise spectra can be described by the normalized noise power, $A$, defined as

$$A = \frac{S_q}{I^2} f \quad (5.4)$$

with $S_q$ the current power spectral density and $I$ the average current. The current noise power resulting from surface charge fluctuations is now given by

$$A = \left( \frac{\partial I}{\partial q} \right)^2 S_q \frac{f}{I^2}. \quad (5.5)$$

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$$S_q = \left( \frac{\sigma (\Gamma - \frac{\sigma}{e})}{\Gamma} \right)^{\gamma^2} f \quad (5.3)$$

where $\gamma$ is a proportionality constant, $F(t)$ is a dimensionless noise function with a $1/f$ power spectrum, and $f$ is the frequency. The term in between brackets in Eq’s. (5.2) and (5.3) expresses the scaling between the surface charge fluctuations and the surface charge density. The fitting parameter $\gamma$ reflects the strength of fluctuations and has the dimension of charge.

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with $S_q$ the current power spectral density and $I$ the average current. The current noise power resulting from surface charge fluctuations is now given by

$$A = \left( \frac{\partial I}{\partial q} \right)^2 S_q \frac{f}{I^2}. \quad (5.5)$$
Assuming a cylindrical nanopore geometry, we can now express the noise power in terms of the surface charge density

\[ A = \left( \frac{1}{\pi d L} \right)^2 \left( \frac{\partial I}{\partial \sigma} \right)^2 S_q \frac{f}{T^2} \]  

(5.6)

where \( d \) is the diameter, and \( L \) is the length of the nanopore. To calculate the noise power, knowledge of the dependency of the ionic current through the nanopore on the amount of surface charge density is thus required.

### 5.2.2 Current fluctuations in solid-state nanopores

We have previously developed a model which successfully describes the conductance through cylindrical nanopores in terms of their surface charge density [7]. The model takes the contribution of the bulk concentration of ions inside the nanopore into account, as well as the contribution of the positive counterions shielding the negative surface charge of the nanopore. Using this expression for the conductance and Eq’s. (5.3) and (5.6), we obtain the current noise power due to surface charge fluctuations:

\[ A = \left( \frac{\sigma (\Gamma - \sigma)}{\Gamma} \right) \left( \frac{\mu + \gamma}{G L^2} \right)^2, \]  

(5.7)

with \( \mu_+ \) for the electrophoretic mobility of the positively charged counterions, and \( G \) the ionic conductance of the nanopore. The model for the nanopore conductance accurately describes the data over salt concentrations spanning 6 orders of magnitude, when the surface reactivity (Eq. (5.1)) is taken into account [8]. The surface charge density is then dependent on the bulk concentration of ions in solution. This dependency was also successfully used for the description of salt-dependent streaming currents in nanochannels [9]. Values of the unknown surface charge density, \( \sigma \), and the total number of chargeable sites per area, \( \Gamma \), follow directly from this model and we adopt parameters from literature [7, 9]. The value of \( \mu_+ \) is given by the mobility of the used potassium ions, \( \mu_+ = 7.6 \times 10^{-8} \) m²/Vs. This leaves the measurable conductance, \( G \), and \( \Gamma / L \) as the only remaining unknown parameters.
5.3 Comparison of models to the experimental data

We now turn to compare the model developed above to measurements of the $1/f$ noise power in solid-state nanopores as a function of salt concentration [4]. First, we look at the nanopore conductance to validate the use of the model and extract the value of the nanopore length $L$. Figure 5.1(a) shows the conductance of three individual nanopores of similar diameter from salt concentrations of 1 mM up to 1.6 M. The conductance does not show a linear decrease upon decreasing the salt concentration, due to the salt-dependent surface charge of the nanopore. The solid line in Fig. 5.1(a) models the conductance, using an average nanopore diameter of $d = 9.3$ nm and a nanopore length of $L = 25$ nm. The calculated conductance gives an excellent description of the data. We can now use the conductance, $G$, and the determined length of the nanopore, $L$, in Eq. (5.7) to calculate the noise power of these nanopores over the salt concentrations probed.

Figure 5.1(b) shows the current noise power of the nanopores as a function of salt concentration. Whereas the conductance decreases when decreasing the salt concentration, the noise power was found to increase. The best fit to the data of the developed surface-charge-fluctuation model (Eq. (5.7)) is shown by the dark grey line in Fig. 5.1(b), where we obtain $\gamma = 9.6 \times 10^{-29}$ C for the single fit parameter.

The surface-charge-fluctuation model predicts an increase in noise power when the salt concentration is decreased. However, the model does not closely follow the trend as measured for the solid-state nanopores. In particular, it fails to give a valid description at high salt concentrations ($\gtrsim 0.1$ M). The noise power is however well described over the whole salt range by Hooge’s phenomenological relation, as shown by the light grey line in Fig. 5.1(b) [4]. Hooge’s relation predicts a linear scaling of the noise power with the inverse number of charge carriers, $N_c$, and is given by $A = \alpha/N_c$. Here, we have used $\alpha = 1.1 \times 10^{-4}$.

5.4 Discussion and conclusions

The surface-charge-fluctuation model developed above can in principle yield a different parameter $\gamma$ for different nanopores. However, its value comparable for all three nanopores analyzed in Fig. 5.1(b). The use of a constant surface charge density for the nanopores does also not improve the comparison to the measurements. Hooge’s relation gives a better description of the $1/f$ low-frequency current noise in solid-state nanopores over the whole salt range probed.
Figure 5.1: Conductance (a) and noise power (b) of three nanopores with similar diameters from salt concentrations of 1 mM up to 1 M. The light grey line in (a) shows the conductance calculated using the model of ref. 7 and the parameters as stated in the text. The dark and light grey lines in (b) are fits to the noise power data from the surface charge fluctuation model and Hooge’s relation, respectively. Hooge’s relation clearly gives the better description of the noise power over the salt range probed.

In the low-salt regime ($\sigma \ll 1/2 \times \text{salt concentration}$) both models obey the same scaling behavior. In this regime the number of charge carriers, $N_c$, and the conductance, $G$, both scale with the surface charge density, $G, N_c \sim \sigma$. Hooge’s relation now directly results in $A \sim 1/\sigma$. The surface-charge-fluctuation model of Eq. (5.7) also gives $A \sim 1/\sigma$, as $\Gamma \gg \sigma/e$. As a result, the validity of the models cannot be discriminated when measurements are performed at low salt concentrations only.

In the high-salt regime ($\sigma \gg 1/2 \times \text{salt concentration}$), the scaling of the models is different. In this regime, $G$ as well as $N_c$ scale with the salt concentration in solution, $[\text{salt}]$; i.e., $G, N_c \sim [\text{salt}]$. As a result, $A \sim 1/[\text{salt}]$ for Hooge’s relation. For the surface-charge-fluctuation model, however, $A \sim \sigma/[\text{salt}]^2$, as still $\Gamma \gg \sigma/e$. This qualitatively explains the fast decrease of the noise power as the salt concentration is increased at high salt concentrations for the surface charge fluctuations model, as observed in Fig. 5.1(b). This fast decrease is not
supported by the measurements.

Summing up, we have developed a model for the low-frequency current noise in solid-state nanopores in terms of surface charge fluctuations. The model is compared to noise data of nanopores at different salt concentrations, as well as with Hooge’s relation. Surprisingly, both models compare equally well to the data at low salt concentrations, as their predicted scaling with salt concentration is identical in this regime. However, the surface charge fluctuation model fails to give a valid description of the data at high salt concentrations. We conclude that the low-frequency noise of solid-state nanopores is best described by the number of charge carriers as expressed in Hooge’s relation and that surface-charge fluctuations are relatively unimportant.

5.5 Acknowledgments

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References

Chapter 5. Comparison of two models for the low-frequency noise
Chapter 6

Nanobubbles in solid-state nanopores

From conductance and noise studies, we infer that nanometer-sized gaseous bubbles (nanobubbles) are the dominant noise source in solid-state nanopores. We study the ionic conductance through solid-state nanopores as they are moved through the focus of an infrared laser beam. The resulting conductance profiles show strong variations in both the magnitude of the conductance and in the low-frequency noise when a single nanopore is measured multiple times. Differences up to 5 orders of magnitude are found in the current power spectral density. In addition, we measure an unexpected double-peak ionic conductance profile. A simple model of a cylindrical nanopore that contains a nanobubble explains the measured profile and accounts for the observed variations in the magnitude of the conductance.

6.1 Introduction

Nanometer-sized pores can be used for the detection of single biopolymers such as DNA or RNA. The charged molecules are electrophoretically driven through a nanopore, resulting in temporal decreases of the ionic current. Translocation measurements have provided a wealth of information on polynucleotides by characterizing DNA contour lengths [1], DNA folding [2, 3], the effective charge of DNA [4], drag on DNA molecules [1], and structural differences [5, 6] of nucleic acids. The technique was first demonstrated using the protein pore α-hemolysin [7], while more recently solid-state nanopores were developed [8, 9]. Fabricated solid-state nanopores have obvious advantages over their biological counterparts, i.e., size control, increased stability, and the potential of device integration. However, they also show undesirable phenomena such as a large variability in conductance [4] and noise [10, 11], both as a function of time and among samples.

In this study, we propose nanometer-sized gaseous bubbles (nanobubbles) as the major source of low-frequency noise and conductance variability. Nanobubbles have been observed in both atomic force microscopy (AFM) [12, 13] and fast freezing experiments [14]. The reason for their thermodynamic stability is still under debate [15]. In our experiments, we scan fabricated solid-state nanopores through a laser focus and simultaneously record the ionic current flowing through the nanopore. The laser acts as a non-invasive probe, creating an equilibrated temperature profile in solution. Upon scanning, single nanopores show dramatic differences in the ionic conductance and low-frequency current noise. An unexpected double-peak conductance profile provides strong evidence for the existence of nanobubbles inside our solid-state nanopores. Our findings provide a framework for improvements on nanopore characteristics. Conversely, they constitute a new probe for the study of nanobubble dynamics.

6.2 Materials and methods

A single nanopore is fabricated in a 20 nm thin SiN membrane covered by 20 nm sputtered SiO$_2$ on each side, as described elsewhere [16]. Figure 6.1(a) shows a transmission electron microscopy image of a nanopore. In our experiments, nanopores with diameters between 5.6 and 12.7 nm are used. Prior to use, the nanopores are treated with an oxygen plasma for 30 s to remove organic contaminants and enhance the hydrophilicity of the surface. The nanopores are mounted onto a home-built inverted microscope with a water immersion objective (NA=1.2, 60×), whose back aperture is filled by a collimated infrared laser ($\lambda$=1064 nm, 1.5 W). The nanopore position relative to the diffraction-limited
6.3 Results

6.3.1 Nanopores moving through a laser focus

Figure 6.1(b) shows the conductance of a 12.7 nm diameter pore as it is moved through the laser focus. The conductance reaches a maximum when the nanopore is situated in the exact focus of the laser and decreases upon moving out of focus. The locally absorbed laser power induces a temperature profile. For the laser powers used, typical temperature differences in the liquid reach maxima of 15 – 30 K. As the nanopore is moved through the laser focus, it acts as a local thermometer [17], since the conductivity of the KCl solution is temperature dependent. The asymmetries in the conductance profile, lower conductance at negative Z relative to positive Z positions, can be attributed to our asymmetric sample layout.
Figure 6.2: Conductance as a function of position relative to the laser focus for two nanopores with a diameter of 5.6 nm (a) and 11.1 nm (b), measured at 500 and 200 mV, respectively. The two lower curves in (a) display a decreased conductance and a dramatic increase in noise. In (b) the conductance instantaneously switches between two (dark grey curve) or three (light grey curve) different values each displaying different noise characteristics. The light grey curve is scanned up to $Z = 0$ only. Current histograms (c) and power spectra (d) show the strong differences in current noise of the curves shown in (b). The histograms are centered around zero by shifting each distribution by $I_0$, the peak value of a Gaussian fit. The dark and light grey histograms are magnified along the Y-axis in order to be visible on the same scale. The inset in (c) shows the total current distribution of the light grey curve. The dark grey curve is analyzed before and after switching to the decreased conductance state. Before switching the dark grey histogram in (c) does overlap with the data shown in black. In (d) the current power spectral density before and after the switching is represented by open and filled dark grey circles, respectively. The low-frequency noise has a $S_f \propto 1/f^\alpha$ frequency dependence with $\alpha = 1.1 - 1.9$. The lines are guides to the eye.
6.3.2 Noise and conductance variability when moving through a laser focus

Surprisingly, scanning an individual nanopore multiple times through the laser focus can result in dramatically different ionic conductance profiles. Figure 6.2(a) and (b) each display three conductance curves, taken when moving a single nanopore through the laser focus. The top curves show a single-peak conductance profile similar to the one shown in Fig. 6.1(b). Figure 6.2(a) additionally shows two curves for which the conductance is significantly reduced for all $Z$ positions. Moreover, both curves show a dramatic increase in the amount of noise. The nanopore can be in a "good" state with high conductance and low noise, or in a "poor" state with a decreased conductance and severely increased noise. In Fig. 6.2(b) we show a nanopore that spontaneously switches between these different states. During the scan indicated by the black curve the nanopore is in the good state. The dark grey curve, however, shows a scan from negative to positive $Z$ positions in which the nanopore instantaneously switches from the good to a poor state. The light grey curve even shows switching between one good and two poor states. Note that all three curves overlap when the nanopore is in the high-conductance low-noise state.

To quantify the differences between these states, Fig. 6.2(c) and (d) show the current histograms and power spectra of the data shown in Fig. 6.2(b). The data shown in dark grey are analyzed in the good and the poor state. The current noise characteristics of the black curve and the good state in the dark grey curve of Fig. 6.2(b) are identical; their histograms and power spectra overlap [see e.g., the black and open dark grey circles in Fig. 6.2(d)]. After switching to the dark grey poor state, however, the width of the current histogram, and consequently the power spectral density, is dramatically increased. The standard deviation of the current histogram of Fig. 6.2(c) increases from 9 pA to 104 pA, and Fig. 6.2(d) shows a difference in power spectral density at low frequencies of more than 2 orders of magnitude. For the light grey curve, the current distribution in Fig. 6.2(c) is even broader. The inset shows all three current levels and the differences in the width of their distributions. Each conductance level shows significantly different noise characteristics and the level that conducts best (at $I - I_0 \approx 2$ nA) clearly shows the lowest current noise. The current noise of the entire light grey curve, is evidently much larger than that measured for the good state (black curve). Figure 6.2(d) shows a difference of 5 orders of magnitude in power spectral density at low frequencies. The current noise levels of tens of nanopores have been recorded at 200 mV, showing similar strong differences from pore to pore, with $S_I(f = 1 \text{ Hz})$ ranging from $10^{-25}$ to $10^{-18}$ A$^2$/Hz (data not...
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In conclusion, we find that nanopores display a large variability in ionic conductance and low-frequency current noise. Moreover, a decreased conductance correlates with a greatly increased amount of noise.

6.3.3 Double-peak conductance profiles

We now turn to a remarkable feature of the measured conductance profiles which provides a clue to the origin of these phenomena. Close inspection of the bottom curve of Fig. 6.2(a) reveals that it does not display the single-peak conductance profile as expected for laser-induced heating. Instead, a surprising double-peak conductance profile is measured. Figure 6.3(a) demonstrates the same effect more clearly in a measurement for a different pore. Here, two typical traces of the conductance are shown for a 7.2 nm diameter nanopore. In the top curve, scanning of the nanopore through the laser focus results in a single conductance peak. The bottom curve of Fig. 6.3(a) clearly shows a reduced conductance for all Z positions and a double-peak conductance profile. As the nanopore is moved from negative to positive Z positions the conductance initially increases, but it starts to decrease at a Z position of approximately $-1 \, \mu m$, reaching a local minimum in the focus of the laser at $Z = 0$. The conductance again increases when the nanopore is moved to positive Z positions, and decreases beyond a Z position of approximately $+1 \, \mu m$. Note that the conductance changes for the two curves of Fig. 6.3(a) are very similar for $|Z|$ positions $> 1 \, \mu m$ from the laser focus. At $|Z|$ positions $< 1 \, \mu m$ from the laser focus, however, the increased local temperature does not result in an increased conductance in the bottom trace. Apparently, a competing mechanism reduces the conductance in this case.

6.4 Modeling of the double-peak conductance profile

We now present a simple model for the ionic conductance through a nanopore as it is moved through the laser focus, which explains the characteristics displayed in Fig. 6.3(a). We assume a simple cylindrical geometry for the nanopore. The laser-induced temperature profile is described by a Gaussian function [18], and since the KCl conductivity has a linear temperature dependence [19], the ionic conductance as a function of laser position is Gaussian as well. The top curve of Fig. 6.3(b) shows the calculated conductance of a nanopore as a function of its position relative to the laser focus. First we calculate the normalized conductance, $G_n(T)$, which is defined to equal 1 when the local temperature equals the
6.4 Modeling of the double-peak conductance profile

Figure 6.3: (a) Two typical scans of the conductance of a 7.2 nm diameter nanopore, measured at 50 mV, as a function of laser position relative to the focus. The bottom curve shows a surprising double-peak conductance profile and a significantly reduced conductance at all \( Z \) positions. (b) Model curves (see text) of the conductance as a function of laser position relative to the focus for different values of the diameter of the nanobubble, \( d_{b0} \), at temperature \( T_0 \). The inset shows a schematic representation of the model. A cylindrical nanopore, with a nanobubble present, moves through the focus of the laser. The nanobubble expands near the focal position as a result of higher local temperatures.

background temperature of the solution \( T_0 \). Subsequently we scale this to the value measured far from the laser focus in the top curve of Fig. 6.3(a). We now insert a spherical nanometer-sized gaseous bubble into the nanopore and again model the conductance as a function of laser focus position. As the nanobubble partially blocks the pore, the conductance of the nanopore is now reduced. Upon moving to the position of the laser focus, the nanobubble will expand as a result of higher local temperatures. The conductance thus increases as a result of an increased conductivity of the KCl solution, but it can also decrease as the size of the nanobubble is increased. The situation is sketched in inset of Fig. 6.3(b). Simply assuming that nanobubble expansion obeys the ideal gas law, the diameter of the nanobubble, \( d_b \), at temperature \( T \) can be written as

\[
d_b(T) = d_{b0} \left( \frac{T}{T_0} \right)^{\frac{1}{3}},
\]  

(6.1)
where $d_{b0}$ is the diameter of the nanobubble at temperature $T_0$. The normalized conductance as a function of temperature follows from simple geometric considerations:

$$
G_n(T) = \frac{1}{R_n(T)} = \frac{\rho(T_0)}{\rho(T)} \left( \frac{L - d_{b}(T)}{\frac{L}{4} d_{pore}^2 + \frac{1}{d_{pore}}} + \int_{0}^{d_{b}(T)} \frac{dz}{A(z)} \right),
$$

(6.2)

where $\rho$ is the resistivity of the solution, $d_{pore}$ and $L$ are the diameter and length of the cylindrical nanopore, respectively, and $A(z)$ is the surface area along the axis of the cylinder. The first term in the denominator on the righthand side of Eq. (6.2) represents the resistance of a cylinder of length $L - d_b(T)$, while the resistance of the remaining cylinder, containing the nanobubble, is given by the second term. The access resistance of the nanopore is taken into account [20]. Figure 6.3(b) shows the conductance as a function of laser position for five different values of $d_{b0}$ [21]. When a nanometer-sized bubble is introduced into the pore, the conductance can be significantly reduced. Evidently, larger nanobubbles result in smaller values of the conductance for all $Z$ positions. For small $d_{b0}$, the conductance monotonically increases as the nanopore is moved towards the position of the laser focus. However, for $d_{b0} = 6.88$ nm the model predicts a strikingly different behavior. Moving the nanopore through the focus of the laser now results in a double-peak conductance profile. Near the focal point of the laser the growing nanobubble gives rise to a decrease in the conductance, as the amount of current blockade due to the size of the bubble grows more rapidly than the conductivity increase of the liquid. The effect is further enhanced for $d_{b0} = 6.96$ nm, resulting in a severely decreased conductance in the focal point of the laser.

6.5 Discussion and conclusions

The model curves presented in Fig. 6.3(b) are remarkably consistent with the data shown in Fig. 6.3(a). The simple model of a nanometer-sized bubble inside the nanopore predicts the measured decreased conductance values for all $Z$ positions as well as the existence of the double-peak conductance profile. Note that a double-peak profile cannot be attributed to the presence of a solid particle inside the nanopore or to nanopore size changes, since the corresponding expansion coefficient is much too small to cause the observed effect [22]. We therefore conclude that a gaseous nanometer-sized bubble is present inside the nanopore. The experimental data in the bottom curve of Fig. 6.3(a) not only show a decreased
conductance but also an increase in the amount of noise, similar to Fig. 6.2. The observed correlation of a decreased conductance and an increase in the amount of noise strongly suggests that the nanobubble also acts as the source of current fluctuations [23].

Hydrophobicity and surface roughness are likely to play a role in the formation and stabilization of nanobubbles. Whereas the diameter of the solid-state nanopores can be controlled accurately, the local surface properties may vary depending on the details of the nanopore formation. We speculate that local surface roughness and hydrophobicity, in combination with trapped air inside the nanopore, result in nanobubble formation [24]. Presumably air is trapped inside the nanopore when the reservoirs on either side are filled with liquid, since degassing our buffer solutions leads to the same observed phenomena. Our notion might explain the noise reduction of nanopores following an atomic layer deposition of the hydrophilic alumina observed by Chen et al. [10]. We suggest that nanobubbles cause a strong increase in the low-frequency current noise due to nucleation, movement along the nanopore surface, and/or dissolution [25].

In conclusion, we observe strong variations in the magnitude of the conductance and the noise for individual nanopores and between pores. Differences in current power spectral density at low frequencies up to 5 orders of magnitude are shown. In addition, we measure an unexpected double-peak conductance profile as the pore is moved through the focus of an infrared laser. A simple model for the ionic conductance through a nanopore with a nanobubble inside compares excellently to the measured data. The essential features cannot be explained by a solid particle partially blocking the pore, but are consistent with a nanometer-sized gaseous bubble inside our solid-state nanopores. The nanobubbles can act as a source of conductance and noise variability. These results not only allow to improve on solid-state nanopore characteristics by addressing the hydrophilicity and surface corrugation of nanopores, but also provide a new tool for studies on nanobubble dynamics.

6.6 Acknowledgements

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References

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[18] A Gaussian temperature profile is assumed with $T_0 = 291.15$ K (the background temperature of the solution) and $T_{\text{max}} = 320.15$ K (the solution temperature at the laser focus position).


[21] The normalized conductance values were calculated using $d_{\text{pore}} = 7.2$ nm and $L = 43.5$ nm. Changing the nanopore geometry merely results in different numerical values.
[22] The expansion coefficients of solids are 3 orders of magnitude smaller compared to those of air. Moreover, TEM and AFM inspection of nanopores after use yielded no traces of contamination.

[23] Note that changes in surface charge and related ion selectivity do not result in any sizeable variation of the conductance at the 1 M salt concentration used [4].


[25] The intrinsic resonance frequency of a nanobubble will be much larger than the frequencies of the current fluctuations under consideration.
Chapter 7

Salt-dependence of ion transport and DNA translocation through solid-state nanopores

We have measured the salt dependence of ion transport and DNA translocation through solid-state nanopores. The ionic conductance shows a three-order-of-magnitude decrease with decreasing salt concentrations from 1 M to 1 µM, strongly deviating from bulk linear behaviour. The data are described by a model that accounts for a salt-dependent surface charge of the pore. Subsequently, we measured translocation of 16.5 µm long dsDNA for 50 mM to 1 M salt concentrations. DNA translocation is shown to result in either a decrease ([KCl] > 0.4 M) or increase of the ionic current ([KCl] < 0.4 M). The data are described by a model where current decreases result from the partial blocking of the pore, and current increases are attributed to motion of the counter ions that screen the charge of the DNA backbone. We demonstrate that the two competing effects cancel at a KCl concentration of 370 ± 40 mM.

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Chapter 7. Salt-dependence of ion and DNA transport

7.1 Introduction

Nanometer-sized pores can serve as single-molecule sensors for the detection and analysis of biopolymers. Sensing of single-stranded DNA (ssDNA) and ssRNA at the single-molecule level was first demonstrated using the protein pore -hemolysin (narrowest constriction of 14 Å) [1, 2]. This protein-pore-based sensor was able to discriminate between different nucleic acid molecules [3, 4], detect single-nucleotide differences [5], and characterise the hybridisation of individual DNA strands [6]. Fabricated solid-state nanopores [7, 8] offer advantages over protein-based pores, such as size control, increased chemical, electrical, mechanical and thermal stability, and the potential of device integration. Solid-state pores have been used to map the three-dimensional intensity profile of a laser [9], detect DNA contour lengths [10], DNA hybridisation [11], DNA folding [12, 13], and the drag [10] on individual DNA molecules. Narrow (1 nm diameter) solid-state pores were also used to filter ssDNA from a solution containing ssDNA and ds-DNA [14]. The physical detection principle of the nanopore sensor is similar to that of Coulter counters which are typically used for the detection of micron-sized particles. The introduction of such a particle into a small capillary results in the partial blocking of the ionic current. As particles are driven through the opening, spike-like decreases in the current can be recorded which are related to the volume of the particles [15]. Nanopore diameters are a thousand times smaller than those of typical Coulter counters and consequently nanopores are capable of detecting objects on the molecular scale. However, at these length scales surface effects may also play a role in the current signal during translocation. The influence of surface charges on ionic transport was recently reported in nanochannels [16–18] and in small diameter synthetic nanopores [19, 20]. DNA translocations through nanopores [21] and nanochannels [22] at isolated low salt concentrations were recently shown to result in current enhancements rather than current decreases. In this letter, we study the salt-dependence of ion transport and dsDNA translocation through solid-state nanopores over a wide range of concentrations. We present ion transport measurements through nanopores over salt concentrations spanning six orders of magnitude. The results are compared to models of bulk behaviour and constant-surface-charge conductance which both fail to describe the data. We introduce a simple model that additionally accounts for the salt-dependent charging of SiO$_2$ and obtain a good description of the data. Furthermore, we present DNA translocations through nanopores over a range of salt concentrations. DNA translocation results in either a decrease or increase of the ionic current. Two competing effects are identified for the current change. We demonstrate the existence of a cross-over point at which DNA translocation
causes no current change. Together, the experiments yield a complete picture of the salt-dependence of DNA translocation through nanopores.

7.2 Fabrication of nanopores and experimental setup

A three-layer structure of 20 nm silicon nitride, 200 nm silicon dioxide and 500 nm silicon nitride was deposited by low-pressure chemical-vapour deposition (LPCVD) on silicon (100) wafers. A thin free-standing membrane of silicon nitride was fabricated by opening a window in the unpolished side of the wafer using electron-beam lithography, followed by reactive ion etching (RIE, CHF$_3$/O$_2$) to remove the deposited layers, after which a KOH wet etch removed the silicon. Next, a window was opened on the polished top side of the wafer, again using electron-beam lithography. The top silicon nitride and silicon dioxide layers were removed with a RIE (CHF$_3$/O$_2$) and a buffered HF etch, respectively. The resulting free-standing silicon nitride membrane was covered with a thin layer of sputtered silicon oxide (approximately 20 nm) on both sides. This results in a hydrophilic membrane surface, while the structure still has the ease of fabrication and good insulation properties of a silicon nitride membrane. Finally, the sample was placed in a transmission electron microscope (TEM) operated at 200 or 300 keV. The use of a tightly focused electron beam on the membrane results in the sputtering of material and hence the creation of a nanopore, as schematically depicted in Fig. 7.1(a). The size of the nanopore can be tuned by using a defocused electron beam with lower intensity which prevents sputtering but does enable surface-tension-driven transport of material [8]. The produced nanopores were directly imaged in the TEM (Fig. 7.1(b)). The diameter of the pores used in these experiments was approximately 10 nm. Information about the three-dimensional geometry was obtained by mapping the thickness variation around the opening using electron energy-loss spectroscopy (EELS) [23]. The EELS results indicate that the length of the narrowest constriction of the nanopore is much smaller than the 60 nm thickness of the membrane, as schematically depicted in Fig. 7.1(c). Prior to inserting the nanopores in the experimental setup for measuring ionic current through the pore, both sides of the sample were subjected to an oxygen plasma for about 15 s. This process removed any organic contaminants and results in the oxidation of the surface (SiO$_2$), which significantly enhances the wettability properties of the nanopores. Next, the patterned chip was placed in between two liquid compartments of a flow cell. One compartment was formed by adhering a patterned polydimethylsiloxane (PDMS) layer to a glass slide, forming
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a channel connecting the sample to fluid inlet and outlet. The other compartment consisted of a channel constructed in a polymethylmethacrylate (Perspex) block which was sealed against the sample with an O-ring. The nanopore formed the only connection between the two compartments. Ag/AgCl electrodes were placed at the outlets of both channels and connected to an amplifier (Axopatch 200B, Axon Instruments, USA). Solutions could be easily exchanged within each compartment by flowing liquid through the channels. Salt solutions were prepared by adding 18 M cm Milli-Q filtered water (Millipore, USA) to a stock solution of 1 M potassium chloride with 20 mM TRIS-HCl buffer at pH= 7.5. Unmethylated 48.5 kbp double-stranded DNA (Promega, Benelux) was used in the translocation studies. The value of the nanopore conductance at various salt concentrations was obtained by sweeping the voltage over a range of 200 mV at a scan rate of approximately 6 mV/s. For DNA translocation, a constant voltage of 120 mV was applied. When DNA was introduced into one of the two compartments, spike-like changes were observed in the current. This current signal was digitised at 200 kHz (Axon 1322A digitiser, Axon Instruments, USA), and low-pass filtered with a cut-off frequency of 10 kHz. When necessary, the data was subjected to an additional external filter of 5 kHz to increase the signal-to-noise ratio. The event-fitting algorithm used to analyse and label the translocation events was similar to the one described by Storm et al [13]. We slightly modified the procedure to determine the open-pore current by averaging the current before and after the event for approximately 2 ms. We have discarded levels lasting shorter than 70 µs (210 µs for the events that were additionally filtered at 5 kHz).

7.3 Experimental results

7.3.1 Salt dependence of the nanopore conductance

Figure 7.2(a) shows an example of current-voltage (I-V) measurements of a single 10 nm pore at five different potassium chloride concentrations. In all experiments, the I-V curves display a linear relationship. Fits to the data yield the value of the pore conductance. Its value clearly decreases as the potassium chloride concentration is lowered. The conductance of ten individual nanopores as a function of the potassium chloride concentration is shown in Fig. 7.2(b). As the salt concentration is varied over six orders of magnitude, the conductance is found to decrease by a factor of ~1000. In the high-salt regime ([KCl] ≥ 100 mM), the conductivity of the nanopores is comparable to the bulk conductivity of KCl. In this regime, the conductance agrees with a linear dependence on potassium chloride concentration as expected for bulk liquid. The lightest grey straight
Figure 7.1: Nanopore fabrication and layout. (a) Schematic of the use of a tightly focussed electron beam on a thin fabricated membrane, resulting in the creation of a nanopore. The material composition of the different layers is indicated in the figure. (b) Transmission electron micrograph of a nanopore (diameter 12 nm) immediately after its formation by the intense electron beam. (c) Schematic cross-section of a fabricated nanopore as inferred from EELS measurements. Only the thin silicon oxide/silicon nitride/silicon oxide membrane is shown. The drilling process results in the removal of material in a broad area around the opening. The indicated angle is approximately 45 degrees.

line indicates the expected bulk behaviour for a cylindrical nanopore of 10 nm in diameter and 34 nm in length. These pore dimensions were used in all calculations; the diameter of the cylindrical model pore is inferred from the TEM images and the choice for the length is justified below. In the low-salt regime ([KCl] < 100 mM) we measure a much larger conductance than expected from bulk behaviour. The data show a gradual decrease in conductance as the potassium chloride concentration is lowered, all the way down to 1 µM.
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Figure 7.2: Salt concentration dependence of nanopore conductance. (a) Current-voltage characteristics for a single 10.2 nm diameter pore at KCl concentrations of 0.01, 0.1, 1, 10, and 100 mM, as indicated. All curves show a linear I-V dependence. (b) Conductance values of ten individual pores measured for KCl concentrations from 1 mM to 1 M. Each individual pore has its own symbol. The black upright triangles represent individual pores that were used at a single salt concentration. All pores used have diameters of 10 ± 2 nm. The lightest, light and dark grey lines show the results of calculations as predicted by bulk behaviour, a model for constant surface charge, and a model for a variable surface charge, respectively (see text). For the calculations, a 10 nm diameter and 34 nm long cylindrical pore was assumed. The inset shows the values of the surface-charge density versus salt concentrations on a log-log scale, for both the constant surface charge model (dark grey, $\sigma = 60 \text{ mC/m}^2$), and the variable-charge model (light grey, parameters are given in Ref. 28).
7.3 Experimental results

7.3.2 DNA translocation through nanopores at various salt concentrations

We now turn to the spike-like changes in the conductance that are observed following the introduction of DNA molecules (Fig. 7.3). The sign of the current changes was found to depend on the salinity of the solution. Figure 7.3(a) shows data of three individual DNA translocation events at a high KCl concentration (500 mM, black). Spike-like decreases in the ionic current are observed that are quantified using an event-fitting algorithm (grey). The current before and after the decrease equals the ionic current in the absence of DNA in the pore. As the three traces demonstrate, not all the DNA translocations show the same magnitude of current decrease. The first and the last trace show a current decrease that differs by approximately a factor of two. The second trace shows two different current levels, each of which is similar to the levels in the two other traces. In this particular case a larger current blockade followed is by a smaller one. Following the nomenclature of Ref. 13, we call this a 21-event, whereas the events on the left and right of Fig. 7.3(a) are called 1- and 2-events, respectively. It was shown that these different types of events result from DNA molecules traversing the pore in different folding conformations where a 1-event is caused by an unfolded DNA molecule and a 21-event by a DNA which enters in a folded state and exits with its unfolded tail [12, 13]. Consequently, the smaller current amplitude blockades exhibit longer translocation times (Fig. 7.3(a)). We next monitored DNA translocation in the same pore ($d_{\text{pore}} = 10.2$ nm), but at a lower KCl concentration of 150 mM. Surprisingly, spike-like current increases were recorded at this low salt concentration (Fig. 7.3(b)). The magnitude of the current increases could again differ by approximately a factor of two, as shown by the first and last trace. The second trace displays two levels of current increases, in this case a large increase followed by a smaller one. We again categorised the traces as 1-, 21-, and 2-events. Again, smaller current amplitude increases were found to correlate with longer translocation times. We now compare these events at the two different potassium chloride concentrations by analysing scatter plots of the change in current versus the time duration of the event. They are presented in Fig. 7.3(c) and 7.3(d) for salt concentrations of 500 mM and 150 mM, respectively. The colour coding reflects the 1-, 21-, and 2-events as identified above. For a KCl concentration of 500 mM (Fig. 7.3(c)), the amplitude of the current decreases range from $\sim 30$ to $\sim 60$ pA from the 1- (black) to 2-events (light grey). The current increases at a KCl concentration of 150 mM range from approximately 60 to 130 pA. In both cases the level of current change for the 2-events is approximately twice as large as for the 1-events. The most probable translocation times for the 1- and
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Figure 7.3: DNA translocation resulting in current decreases at 500 mM, and current enhancements at 150 mM KCl. (a) Examples of three individual DNA translocation events at KCl concentrations of 500 mM and (b) 150 mM. The data in (a) and (b) were measured using the same pore of 10.2 nm diameter. Data shown in black was additionally filtered at 5 kHz. The grey dashed lines display the result of the event-fitting algorithm. The traces displayed show subsequent translocation of unfolded, partially folded and fully folded DNA molecules from left to right, respectively. (c) Event scatter diagram of the amplitude of the current change versus translocation time for the salt concentration of 500 mM. (d) Same for 150 mM. Colour coding represents translocation of unfolded (black), partially folded (grey) and fully folded (light grey) DNA molecules.

2-events are, respectively, 1.2 ± 0.1 ms and 0.48 ± 0.05 ms for a KCl concentration of 500 mM, and 1.4 ± 0.1 ms and 0.45 ± 0.04 ms for 150 mM [24]. Clearly, the characteristics of the spike-like ionic current enhancements are strikingly similar to those of the current blockades, with the obvious exception that the sign of the current change is reversed. From the similarities between the distinct current levels and the translocation times, we infer that the recorded current enhancements are caused by DNA translocation through the nanopore.

We subsequently measured translocation events over a range of salt concentrations (1 M down to 50 mM, Fig. 7.4(a)). Attempts to measure DNA translocation...
at 1 mM and 10 mM KCl concentrations did not yield any translocation events. For simplicity, only unfolded type-1 DNA translocation events were considered in the analysis. The conductance changes due to DNA translocation were found to gradually change from blockades to enhancements as the KCl concentration was lowered from 1 M [25]. Moreover, the conductance change appears to be linearly proportional (solid line) to the concentration of ions in the bulk solution. The linear fit yields a cross-over point, at which current decreases change to current enhancements, at a KCl concentration of 370 ± 40 mM. In principle, the conductance change, $\Delta G$ depends on the length of the nanopore. Because of potential geometrical variations from pore to pore, one might argue that it is more reasonable to consider the relative change in conductance, $\Delta G/G$, because its value no longer depends on the length of the nanopore, but only on its diameter, which is known from TEM imaging. In Fig. 7.4(b), we have plotted the relative conductance change as a function of potassium chloride concentration. Again, a transition from a decreasing to an increasing change in conductance is observed. The relative change in conductance is much higher in the low-salt regime, which is caused by the lower conductance, $G$, of the nanopore. The values of $\Delta G/G$ show considerable scatter at a KCl concentration of 0.1 M, whereas the values of $\Delta G$ (Fig. 7.4(a)) are similar. This scatter is caused by variations in the conductance, $G$, for different nanopores of similar size (Fig. 2b). The most probable DNA translocation time as a function of potassium chloride concentration is shown in the inset of Fig. 7.4(b). Within the experimental error, the translocation time appears to be constant over the KCl concentrations probed. As the translocation time is directly related to the electrophoretic mobility of the DNA, the result suggests that the electrophoretic mobility of DNA is constant for KCl concentrations from 50 mM to 1 M.

7.4 Modelling and Discussion

7.4.1 Salt dependence of the nanopore conductance

Because of the high surface-to-volume ratio in nanopores, surfaces potentially have a large effect on the conductance at lower salt concentrations. We now discuss how the nanopore conductance is influenced by surface charges in the pore. At high salt concentrations, we expect charge carriers in the solution to dominate the ionic current. The conductance should then scale linearly with the number of charge carriers, as observed experimentally (Fig. 7.2(b)). The negative surface charge of the SiO$_2$ nanopore is screened by mobile $K^+$ counterions that contribute to the overall ionic current. The total current through the nanopore is
Figure 7.4: Conductance changes (a) and relative conductance changes (b) due to DNA translocation for KCl concentrations between 50 mM and 1 M. Only unfolded (type-1) DNA translocations are analyzed. Each symbol represents an individual pore of diameter 10 ± 2 nm except for the grey right (15 nm) and left (21 nm) pointing triangles. The solid line in (a) represents a linear fit to the data. The solid line in (b) represents calculated values upon taking a changing surface charge into account and using the values obtained from the fit in (a) (see text). The inset in (b) shows the DNA translocation time as a function of KCl concentration.

Therefore equal to the sum of the contributions of the bulk concentration of ions in solution and the counter ions shielding the surface charge. The conductance, $G$, can be written as

$$G = \frac{\pi}{4} \frac{d_{\text{pore}}^2}{L_{\text{pore}}} \left( (\mu_k + \mu_{cl}) n_{kcl} e + \mu_k \frac{4\sigma}{d_{\text{pore}}} \right)$$

where $d_{\text{pore}}$ represents the diameter, $L_{\text{pore}}$ is the length of a cylindrical nanopore, $n_{kcl}$ is the number density of potassium or chloride ions, $e$ is the elementary charge, $\sigma$ is the surface-charge density in the nanopore, and $\mu_k$ and $\mu_{cl}$ are the electrophoretic mobilities of potassium and chloride ions, respectively. We used
values of $\mu_k = 7.616 \cdot 10^{-8} \text{ m}^2/\text{Vs}$ and $\mu_{cl} = 7.909 \cdot 10^{-8} \text{ m}^2/\text{Vs}$ [26]. The first term in Eq. (7.1) represents the bulk conductance, while the surface charge contribution to the conductance in the nanopore is given by the second term. At KCl densities higher than $n_{kcl} \gg 2\sigma/d_{pore} e$, the first term in the formula dominates the conductance and bulk behaviour is observed. Deviations from bulk behaviour start to occur when the first and the second term in Eq. (7.1) are comparable. As $n_{kcl}$ is lowered further, surface effects govern the nanopore conductance. For a pore with a diameter of 10 nm and a value of the surface charge of 60 mC/m$^2$, as was found for SiO$_2$ nanochannels [16], the cross-over concentration is estimated at 120 mM. Indeed, the deviations from bulk behaviour were found to occur near this concentration (Fig. 7.2(b)). Equation (7.1) now gives the conductance as a function of potassium chloride concentration (darkest grey line in Fig. 7.2(b)). In the high-salt regime, both the model and the experimental data show the linear bulk behaviour. However, in the low-salt regime, the use of a constant surface-charge density results in a constant conductance. This clearly contradicts our experimentally observed behaviour of a gradually decreasing conductance with decreasing KCl concentration. However, the surface charge is not constant but should depend on the ion concentration. This follows from the chemical reactivity of the silicon dioxide surface given by

$$SiOH \rightleftharpoons SiO^- + H^+ \quad (7.2)$$

Assuming thermodynamic equilibrium, the concentration of $H^+$ ions near the surface is set by the local electrostatic potential. This potential drives the equilibrium of the chemical reaction and hence determines the amount of surface charge present. Behrends and Grier [27] derived a relationship between the potential at the no-slip plane ($\zeta$ potential) and the surface charge density, $\sigma$, taking into account surface reactivity

$$\zeta(\sigma) = \frac{k_BT}{e} \ln \left( \frac{-\sigma}{e\Gamma + \sigma} \right) + \frac{k_BT \ln(10)}{e} (pK - p\text{H}) - \frac{\sigma}{C} \quad (7.3)$$

where $k_BT$ represents the thermal energy, $\Gamma$ is the surface density of chargeable sites, $pK$ is the equilibrium constant, and $C$ is the capacitance of the Stern layer. An additional relationship between $\zeta$ and $\sigma$ is given by the Grahame equation, which couples the electrostatic potential and the charge in the diffusive layer

$$\sigma(\zeta) = \frac{2\epsilon\epsilon_0 k_BT \kappa}{e} \sinh \left( \frac{e\zeta}{2k_BT} \right) \quad (7.4)$$
where $\epsilon_0$ denotes the permittivity of the solution and $\kappa^{-1}$ the Debye screening length (equal to $\kappa^2 = 2e^2n_{kcl}/k_BT\epsilon_0$). Combining Eqs. (7.3) and (7.4) yields the surface charge as a function of the potassium chloride concentration. Recently, this model was successfully used by van der Heyden et al. to model streaming currents in SiO$_2$ nanochannels as a function of salt concentration [28]. In the inset to Fig. 7.2(b), we plot $|\sigma|$ as a function of potassium chloride concentration using the parameters given in ref. 28. When taking into account the chemical reactivity of the surface, the surface-charge density shows a monotonic decrease by more than 1 order of magnitude.

The varying surface charge obtained can be substituted into Eq. (7.1) to determine the salt-dependent conductance of a nanopore. The result is shown by the middle grey line in Fig. 7.2(b). The dependence of the surface charge on the potassium chloride concentration, as predicted by the chemical equilibrium model, has a large impact on the nanopore conductance. As mentioned before, the conductance is dominated by bulk behaviour in the high salt regime. However, for potassium chloride concentrations below $\sim 100$ mM, the calculated conductance strongly deviates from both the bulk behaviour and from the model that assumes a constant surface-charge density. The simple model presented here is remarkably consistent with the experimentally observed concentration dependence of the conductance. Upon adopting the parameters from literature and without the need of any fitting parameters, the model excellently describes the gradual decrease in conductance as the potassium chloride concentration is varied over the full range of salt concentrations.

### 7.4.2 DNA translocation through nanopores at various salt concentrations

The salt dependence of DNA translocation is addressed with a model that considers two competing effects. On the one hand, the conductance is decreased due to the volume that is occupied by the DNA [29, 30]. In other words, the introduction of the DNA strand into the nanopore affects the bulk conductance by decreasing the number of charge carriers available for ionic transport. On the other hand, the counter ions shielding the charge of the DNA backbone add a positive contribution to the ionic current. The DNA molecule introduces a cloud of mobile counter ions into the pore, thereby increasing the number of charge carriers available for ionic transport [21, 22]. Taking both effects into account we
can express the change in conductance, $\Delta G$ due to DNA translocation as

$$\Delta G = \frac{1}{L_{\text{pore}}} \left( -\frac{\pi}{4} d_{\text{DNA}}^2 (\mu_k + \mu_{cl}) n_{kcl} e + \mu_k^* q_{l,DNA}^* \right)$$

(7.5)

where $d_{\text{DNA}}$ (2.2 nm) represents the diameter of the molecule, $\mu_k^*$ is the effective electrophoretic mobility of potassium ions moving along the DNA, and $q_{l,DNA}^*$ is the effective charge on the DNA per unit length which is assumed to be constant. Since the left-hand term, expressing the change in bulk conductance, depends on $n_{kcl}$, we can expect a linear relationship for $\Delta G(n_{kcl})$ (as indeed observed experimentally, see Fig. 7.4(a)) and, hence, a crossover point at which $\Delta G = 0$.

Free parameters $\mu_k^* q_{l,DNA}^*$ and $L_{\text{pore}}$ in Eq. (7.5) can be obtained from the linear fit describing the data (Fig. 7.4(a)). We obtain $\mu_k^* q_{l,DNA}^* = (2.09 \pm 0.06) \times 10^{-17}$ m/Ω and $L_{\text{pore}} = 34 \pm 2$ nm. The error denotes the standard deviation resulting from the fitting procedure. The value found for the length of a nanopore appears reasonable, considering the geometrical considerations sketched in Fig. 7.1(c) [31]. We can compare the effective electrophoretic mobility of potassium ions and the effective charge on the DNA per unit length to values reported in literature. If we assume that $\mu_k^*$ equals the bulk ionic mobility, $\mu_k$, we can extract an effective charge on the DNA per unit length of $q_{l,DNA}^* = 0.58 \pm 0.02$ electron charges per basepair. This indicates a charge reduction of $71 \pm 2\%$ of the bare charge of $2e^-$ per basepair. Values reported in the literature for the effective charge on the DNA have previously been extracted from indirect measurements and vary widely [32–34]. With a direct measurement, the effective charge of the DNA was recently determined by Keyser et al. [35], which yielded $q_{l,DNA}^* = 0.53 \pm 0.05$ electron charges per basepair, a value that compares well to the value reported here. Alternatively one could assume a charge on the DNA of $2e^-$ per basepair and extract a reduced value for the effective mobility of the counter ions of the DNA equal to $(2.22 \pm 0.06) \times 10^{-8}$ m$^2$/Vs.

The models developed above for $G$ and conductance changes, $\Delta G$, due to DNA translocation can be compared to the measured relative conductance change, $\Delta G/G$ (Fig. 7.4(b)). Because of the limited salt range probed, the data do not discriminate well between models of constant or salt-dependent surface-charge density. We combine Eqs. (7.1), (7.3), (7.4), and (7.5) to calculate the expected $\Delta G/G$. This is shown in Fig. 7.4(b) by the solid grey line, using the same parameters as van der Heyden et al. [28] and the product of $\mu_k^* q_{l,DNA}^*$ found above. The model gives a quite good description of the data. [36] We attribute part of the variation between nanopores with similar diameters to a variation in surface charge. We conclude that the absolute change in conductance is the
better parameter for characterizing translocations.

### 7.5 Conclusions

We have measured the ionic conductance through nanopores over a salt range of six orders of magnitude. A strong deviation from bulk behaviour was found for potassium chloride concentrations lower than 100 mM. A much more gradual conductance decrease than expected from bulk models was measured for salt concentrations from 100 mM down to 1 µM. We show that the salt-dependent surface charge of 10-nm-diameter pores contributes significantly to the ionic current at these salt concentrations. A surface charge adapting according to the local chemical equilibrium, is supported by the data.

The change in conductance during DNA translocation was observed to change from decreasing to increasing values as the potassium chloride concentration is lowered. In both cases, DNA folding and similar translocation times were recorded. We measured this change over a range of salt concentrations and determined the transition point where DNA translocation has no net effect on the ionic current. A simple model links the decreases in conductance to the volume occupied by the DNA, and the increases in conductance to the mobile counter charge of the DNA molecule. These experiments show that DNA translocation can be used to estimate the DNA volume and obtain a measure of its effective charge. This provides an extension of the Coulter counter principle, and illustrates that nanopores may serve as a new tool to measure the dynamics and effective charge of polymers, proteins, and DNA-protein interactions.

### 7.6 Acknowledgements

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### References


References


The most probable translocation time is the time associated with the peak in the distribution. The error that is quoted is dominated by temperature variations in the setup. A variation of about 3 degrees leads to an estimated error of 10 percent (cf. ref 11).

At a KCl concentration of 300 mM the traces occasionally show events that were composed of a first part of current blockades and a second part of enhancements. Since the current enhancements dominated the behaviour and no current blockades events were recorded, only the current enhancements were analysed.

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The value found here was also used for calculations on the salt dependence of the nanopore conductance as discussed in relation to Fig. 7.2.


The one data point at 100 mM, which has a lower value of the relative change in conductance, was measured with a 20 nm diameter pore whereas the grey line was calculated for a pore diameter of 10 nm.
Chapter 8

Translocation of RecA-coated dsDNA through solid-state nanopores

We report on the translocation of fully protein-coated double strand (ds)DNA molecules through solid-state nanopores. We use the recombination protein A (RecA) to form stable nucleoprotein filaments on DNA, and we confirm their full coverage by atomic force microscopy (AFM). Translocation measurements on the nucleoprotein filaments show large current blockades events, as compared to bare dsDNA, with a wide variety in time duration ($\approx 10^{-4} - 10^{-1}$ s) and conductance blockade values ($2.5 - 14$ nS). The events can be well classified in two distinct groups, based on the value of their conductance blockade. From measurements on different nucleoprotein filament lengths we identify the events with high conductance blockades ($11.9 \pm 0.5$ nS) with the translocation of dsDNA coated with RecA. We estimate the diameter of the nucleoprotein filament by comparing these conductance blockades to measurements on bare dsDNA, and we obtain 8.5 nm, in good comparison with the available structural data. Furthermore, we also analyze the rate of RecA-coated dsDNA current blockade events as a function of voltage, and find two different regimes that cross over at 150 mV. In the low-voltage regime, the event rate increases exponentially with voltage, whereas in the high-voltage regime, it remains constant.

Chapter 8. Translocation of RecA-coated DNA molecules

8.1 Introduction

Nanopores can be used as single-molecule sensors to detect the passage of DNA, RNA, or proteins. The charged biomolecules are driven through the nanometer-sized constriction by an applied electric field. Translocation through the nanopore results in a transient current blockade, a consequence of the volume displaced by the passing of the molecule. Both biological pores, in particular α-hemolysin, and fabricated nanopores have been used to obtain dynamic and structural information on polynucleotides [1]. Polypeptides were studied by translocation through nanopores [2, 3], or by sensing DNA-protein rupture where the nanopore acts as a local force actuator to pull the two molecules apart [4–7]. Here, we use the recombination protein A (RecA), which is known to form stable nucleoprotein filaments on dsDNA [8], to investigate translocation of protein-coated DNA. The RecA protein plays a central role in homologous recombination in prokaryotes as it promotes DNA strand exchange reactions. Using nanopores for the identification of RecA-coated regions along DNA strands may provide for a scheme of homologous sequence detection of target DNA analogous to previously performed atomic force microscopy studies [9], but now directly in liquid and at high speed. In this study, we present translocation measurements of fully RecA-coated double-strand (ds)DNA molecules. We first demonstrate the formation of complete RecA-dsDNA structures by atomic force microscopy (AFM), and subsequently show that translocation measurements result in large current signals compared to bare dsDNA molecules. We analyze the recorded events by their time duration and conductance blockade, and we find large variations in both parameters. We separate the events in two groups, based on their conductance blockade, and show that the time duration of the group with high conductance-blockade values depends on the length of the nucleoprotein filament. We identify these as translocations of RecA-coated DNA. We compare these blockade events to measurements on bare dsDNA and deduce a nucleoprotein filament diameter of 8.5 nm in good agreement with the expected value. Furthermore, we present measurements on the event rate of RecA-dsDNA filaments as a function of voltage and identify two different regimes that cross over at a voltage of 150 mV. These results are the first reported measurements on translocation of protein-coated DNA structures. They not only provide a framework to identify sequences on a target DNA strand, but also form a starting point to address local DNA-protein structures using solid-state nanopores.
8.2 Solid-state nanopores

Solid-state nanopore fabrication starts with the use of standard microfabrication technology to create free-standing membranes. The manufactured 20 nm thin low-stress silicon nitride (SiN) membranes are mostly covered by a layer of 20 nm sputtered silicon oxide (SiO$_2$), on both sides. In each membrane we fabricate a single nanometer-sized pore through the use of a highly focussed electron beam in a transmission electron microscope (TEM). Subsequently, the diameter of the fabricated nanopore can be tuned by exposure to a de-focused electron beam with lower intensity. Details of the fabrication process are described elsewhere [10]. Figure 8.1(a) shows a TEM image of a fabricated solid-state nanopore of 23.3 nm diameter. In this study, we use nanopores with diameters from approximately 20 to 35 nm with good low-frequency noise properties [11]. All nanopores are treated in an oxygen plasma for about 30 s prior to use. Subsequently, the nanopores are mounted in a polyetheretherketone (PEEK) microfluidic flow cell, and sealed to liquid compartments on either side of the sample. Both compartments provide easy access for the placement of electrodes and exchange of solutions. Ag/AgCl electrodes are used to detect ionic currents and apply electrical fields. The currents are detected using a resistive feedback amplifier (Axopatch 200B, Axon Instruments) and low-pass filtered at 10 kHz before digitization at 100 kHz. The experiments are performed in a 1 M KCl salt solution containing 50 mM TRIS-HCl, 1 mM EDTA and 3.1 volume % glycerol at pH= 7.9. The glycerol is added to ensure equal conditions in both compartments upon addition of the nucleoprotein filament to a single compartment. A schematic of the experimental lay-out is shown in Fig. 8.1(b).
8.3 Nucleoprotein filament formation

Figure 8.2(a) shows a tapping-mode atomic force microscopy (AFM) image (Nanoscope IIIa, Digital Instruments, Santa Barbara, CA, USA) of 20 kbp ds-DNA. The images are made in air after depositing DNA from an imaging buffer containing 10 mM MgCl$_2$ and 5 mM TRIS at pH = 7.9 on freshly cleaved mica. The DNA molecules have an apparent height of $Z \leq 0.5$ nm, as is evident from the line scan shown in Fig. 8.2(a). Note that the $Z$ is lower than the expected value from the DNA crystal structure, as is commonly observed in AFM imaging. We coat lambda-DNA molecules (or DNA molecules of smaller length) with the RecA protein by subsequently adding lambda DNA (Promega, WI, USA), RecA (New England Biolabs, MA, USA), and ATP$\gamma$S (Roche, Switzerland) to achieve final concentrations of approximately 1.2 nM, 6.5 $\mu$M, and 1.5 mM, respectively. The ATP$\gamma$S is required as RecA binding to dsDNA only occurs in its active state, which is obtained by the binding of the ATP analogue. Moreover, filaments formed in the presence of the ATP$\gamma$S are known to be very stable [12]. The final solution contains 100 mM NaCl, 10 mM MgCl$_2$, and 5 mM TRIS-HCl and is incubated for 1 h at 37 $^\circ$C. The ratio of RecA protein versus DNA base-pairs was varied to obtain fully coated DNA molecules with a low background of free proteins. Figure 8.2(b) shows an AFM image of the resulting nucleoprotein filaments. From the line scan along the direction indicated by the black line in Fig. 8.2(b), we find an apparent height of $Z \approx 2.5$ nm for the DNA/protein complexes. This is clearly much larger than the value measured for the bare dsDNA molecules, shown in Fig. 8.2(a). We observe the increased height along the total length of the DNA, indicating full coverage. The formed nucleoprotein filaments are stable for hours even in a 1 M KCl salt solution, as confirmed by subsequent AFM imaging (data not shown). The result obtained compare well with previously performed AFM studies on RecA-dsDNA complexes, which show comparable filaments with a height ranging from 2.5 to 4.4 nm [13–16].

To date, no high-resolution structure of RecA in the presence of DNA and ATP($\gamma$S) is available. However, much is known about the structure of the RecA protein [17], and many structural details of RecA filaments formed on dsDNA have been resolved by electron microscopy [18]. These studies show that the nucleoprotein filament is a right-handed helical structure composed of 6.2 RecA units per turn over a pitch of 9.5 nm. The dsDNA is bound within this filament with approximately one RecA monomer bound per three duplex base pairs, resulting in a 1.5 fold DNA extension [19]. This means that a lambda-DNA molecule will be extended to about 25 $\mu$m upon full coating with RecA. The values of the persistence length and the diameter of the filaments are much larger then those.
8.4 RecA-coated dsDNA translocation

Figure 8.2: Atomic force microscopy (AFM) images of (a) 20 kbp dsDNA and (b) RecA-coated λ-DNA on mica. The bottom of both images contains a recorded height profile along the line indicated in black.

of naked dsDNA. The persistence length is increased upon RecA coating to a value of approximately 950 nm [20], and the nucleoprotein filament appears as 10.0 nm diameter rods in electron microscopy. Hegner et al. found a value of 9.2 nm [20], by averaging 12.0 nm diameter cross-sections of the crystal structure of a presumably inactive filament [21].

8.4 RecA-coated dsDNA translocation

Figure 8.3(a) shows current recordings of a 31.1 nm diameter nanopore at 120 mV before and after the addition of bare dsDNA and RecA-coated dsDNA molecules. When no molecules are present in solution, the ionic current recording displays a stable baseline with no significant deviations to lower or higher current values. Upon addition of λ-DNA molecules to the negatively biased compartment, short temporal current blockades appear. These blockades can be seen to reduce the ion current through the nanopore to 100 – 300 pA lower values. Subsequently, we add 5 kbp RecA-coated dsDNA filaments to the same compartment, also resulting in temporal current blockades. However, these current deviations differ substantially from the blockades measured for the bare dsDNA, reducing the current to approximately 1000 – 1500 pA lower values. The much larger current block-
ades measured for the RecA-coated dsDNA compared to the bare dsDNA, are expected because of its larger diameter. Its translocation from the negatively to the positively biased electrode indicates an overall negative charge, in agreement with the isoelectric point (∼5.6) of the purified RecA protein [8].

We now analyze the translocation events of RecA-coated dsDNA molecules according to their conductance blockade and time duration. All events with conductance blockades that exceed the nanopore current noise are taken into account. The value of the conductance blockade is determined with respect to the open-nanopore conductance and represents the average blockade over the time duration of the event. The open-pore conductance is simply the average conductance (over ∼2.5 ms) before and after each translocation event and we define that events begin and end at conductance values one standard deviation from this average.

Figure 8.3(b) shows the conductance blockade versus time duration of RecA-coated λ-DNA translocation events in a semi-logarithmic scatter plot. The measurements are performed using a 23.3 nm diameter nanopore (shown in Fig. 8.1(a)) and at a bias voltage of 180 mV. The events show a large variation in both the conductance blockade and the event duration, ranging from approximately 2.5 to 14 nS, and from 10^{-4} to 10^{-1} s. We classify the events in two distinct groups, indicated by the dotted areas in Fig. 8.3(b). Most events either show a relatively low (∼4 nS, black) or large (∼13 nS, grey) conductance blockade, as is also evident from the histograms shown in Fig. 8.3(c). The duration of events with relatively small blockade values ranges from 10^{-4} to 10^{-3} s. In contrast, the RecA-coated dsDNA events of large conductance blockades show a larger spread in event duration with values ranging from 5 \times 10^{-4} to 5 \times 10^{-2} s. We characterize the events of the two groups by their most probable conductance blockade and time duration. These values are obtained from fitting a Gaussian and log-normal distribution to the conductance blockade and event duration histograms as shown in Fig. 8.3(c) and (d), respectively. We find \( t = 237 \pm 3 \) µs and \( \Delta G = 4.3 \pm 0.1 \) nS for the events with low conductance blockades, and \( t = 2.1 \pm 0.2 \) ms and \( \Delta G = 12.6 \pm 0.03 \) nS for the events showing large conductance blockades, where the error bars denote the errors in the mean.

We now identify the distinct event groups as either resulting from RecA-coated dsDNA molecules that temporarily block the nanopore, or from the actual translocation through the nanopore. Figure 8.4(a) and (b) shows contour plots of the event duration versus conductance blockade of 5 kbp and 48.5 kbp RecA-coated dsDNA respectively. We can again clearly identify two distinct groups of events for the data presented in Fig. 8.4(a) and (b), which can be easily separated by their conductance blockade value. We can again characterize the events
8.4 RecA-coated dsDNA translocation

Figure 8.3: (a) Current recording of a 31.1 nm diameter nanopore at 120 mV before and after the addition of bare λ-DNA and 5 kbp dsDNA/RecA filaments to the negatively biased electrode. Time and current scales as well as the molecules addition are indicated. Clear current deviations from the baseline appear upon addition of the molecules, with much larger current blockades for the filaments as compared to the bare dsDNA. (b) Event scatter plot of time duration vs. conductance blockade of RecA-coated dsDNA translocation events and their respective histograms (c,d). The dotted circles indicate two distinct groups of events. We analyze the events as having either a low (< 10.3 nS, black) or high (≥ 10.3 nS, grey) conductance blockade, and the made separate histograms for each group as shown by the color coding in (c,d).
Chapter 8. Translocation of RecA-coated DNA molecules

of each group by their most probable conductance blockade and time duration. We obtain values of $t = 224 \pm 7 \mu s$, $\Delta G = 6.9 \pm 0.1$ nS and $t = 870 \pm 90 \mu s$, $\Delta G = 11.2 \pm 0.2$ nS for the two groups of the 5 kbp long construct presented in Fig. 8.4(a). For the 48.5 kbp long construct shown in Fig. 8.4(b) we find $t = 273 \pm 10 \mu s$, $\Delta G = 7.3 \pm 0.1$ nS, and $t = 1.9 \pm 0.1$ ms, $\Delta G = 11.6 \pm 0.1$ nS. When comparing the data obtained on different nucleoprotein filament lengths, we notice that the time duration of the high-conductance blockade events depends on the filament length, whereas the time duration of the low-conductance blockade-events is clearly length independent. As the time duration of translocation events is expected to scale with the length of the construct, we conclude that the high-conductance blockade events represent translocation events of RecA-coated dsDNA. We speculate that the lower-conductance-blockade events are due to molecules that arrive at the nanopore and temporarily block the pore but do not translocate. This agrees with the observed lower conductance blockade and time duration of these events compared to the high-conductance-blockade events. Given the fact that the RecA-coated DNA has a factor $\approx 20$ larger persistence length than bare DNA, one may speculate that these low-conductance blockades are due to filaments that approach the nanopore in a perpendicular orientation and temporarily block the pore rather than translocate the nanopore in a parallel fashion.

We can now analyze the measured RecA-coated $\lambda$-DNA translocation events and compare them to the well studied translocation of bare $\lambda$-DNA. Unfolded, head-to-tail translocation events of bare $\lambda$-DNA show most probable translocation times and conductance blockades of approximately $1.3 \pm 0.4$ ms (at 120 mV) and $0.8 \pm 0.1$ nS, respectively [22]. The average values of the RecA-coated $\lambda$-DNA translocation events are larger (see Fig. 8.4(b)), with translocation times of approximately $2.0 \pm 0.2$ ms (at 120 mV) and conductance blockades of $11.9 \pm 0.5$ nS. Furthermore, the spread in translocation times is also very different. Whereas for the bare dsDNA the translocation time can be fitted to a Gaussian on a linear scale, the event time duration of the RecA-coated dsDNA is fitted to a log-normal distribution (i.e., a Gaussian on a logarithmic scale) over two decades.

Longer translocation times for the nucleoprotein filament as compared to the bare dsDNA (2.0 ms versus 1.3 ms) are expected as recA binding along the total DNA length results in a 1.5 fold extension of the molecule. The measured translocation times nicely match this predicted 1.5 fold increase. However, the good comparison between the values might be coincidental. The nucleoprotein filament has an increased persistence length and possibly a decreased effective charge as compared to the naked DNA. As a result, both the driving and the dominant counteracting force might be different [23]. Un-tanglement of these
8.4 RecA-coated dsDNA translocation

![Figure 8.4](image)

**Figure 8.4**: Event duration versus conductance blockade of RecA-coated (a) 5 kbp and (b) 48.5 kbp dsDNA, presented as contour plots. The data is recorded at 120 mV, using nanopores of 31.1 and 24.2 nm diameter, respectively. Both contour plots show two distinct groups of blockade events. Only the event time duration of the higher-conductance-blockade group clearly depends on the length of the nucleoprotein filament, which indicates that these events result from nucleoprotein filament translocation.

Moreover, the value of the translocation time of the RecA-coated dsDNA may also differ due to intrinsic reasons. The large spread in the measured translocation time values of the RecA-coated dsDNA is similar to that found in experiments performed on protein-DNA rupture through an α-hemolysin protein nanopore [4]. In these experiments, the nanopore acts as a local force actuator pulling the protein-DNA complex apart after which only the latter can proceed to translocate through the nanopore. As a result, the resulting time duration of these events shows a very broad distribution over almost two decades, similar to duration distributions shown in Fig. 8.3(b). We however confirmed translocation of the RecA-coated dsDNA through the nanopore by recapture experiments (data not shown), a method which was published recently [24]. Therefore, we conclude that the long translocation times measured for the nucleoprotein filament most probably reflect protein-nanopore interactions during translocation that oppose...
the electrophoretic velocity of the coated DNA molecule.

The most probable conductance blockade of the nucleoprotein filament is about 15 times larger than the blockade measured for the bare dsDNA (11.9 nS versus 0.8 nS). The value of the conductance blockade results from a combination of the volume of the translocating molecule and its effective charge [22]. We can crudely estimate the diameter of the nucleoprotein filament by neglecting the effect of the charge of the molecule (high-salt approximation), which gives \( \Delta G_{\text{RecA}}/\Delta G_{\text{DNA}} = (d_{\text{RecA}}/d_{\text{DNA}})^2 \), with \( \Delta G_{\text{RecA}} \) and \( \Delta G_{\text{DNA}} \) the conductance blockade, and \( d_{\text{RecA}} \) and \( d_{\text{DNA}} = 2.2 \) nm the diameter of the nucleoprotein filament and the bare dsDNA molecule, respectively. We find a value of 8.5 nm for the diameter, which represents the average cross-section of the active helical nucleoprotein filament. The obtained value compares well to the average cross-section of 9.2 nm determined for the inactive filament [20], and to the 10.0 nm diameter of the enveloped active filament as determined from electron microscopy [18].

8.5 Rate of RecA-coated dsDNA events

We now report and analyze the number of events in a given time interval, the event rate, for the RecA-coated dsDNA filaments as a function of the applied voltage. Figure 8.5(a) shows 10 seconds-long current-time traces of RecA-coated dsDNA translocation measurements at five different voltages. The number of current blockade events clearly depends on the voltage, varying from 0.5 ± 0.2 events per second at 80 mV to 11.6 ± 0.4 events per second at 160 mV.

We can describe the occurrence of blockade events by a Poisson process [25]. The probability of observing no RecA-coated dsDNA blockade events, \( P_0 \), during a time interval \( t \) is then given by

\[
P_0(t) = e^{-Rt},
\]

where \( R \) denotes the event rate. We can now determine \( P_0 \) versus \( t \) by analyzing the time between successive translocation events \( \delta t \). We use a constant nucleoprotein filament concentration and only analyze events which reach a conductance blockade \( \geq 5 \) nS. Plotting the \( \delta t \) values in a histogram immediately gives the probability distribution to observe an event after a specific elapsed time \( t \). From this we simply obtain the probability \( P_e \) to observe an event after a time \( t \), by addition. The resulting \( P_0 \) versus \( t \) now follows from \( P_0 = 1 - P_e \).

Figure 8.5(b) shows the obtained probability density distribution \( P_0 \) of RecA-coated-dsDNA events versus time for five different voltages. All distributions
show clear exponential behavior, with values ranging from $R = 2.3 \pm 0.2 \text{ s}^{-1}$ to $R = 13.9 \pm 0.4 \text{ s}^{-1}$ for 120 to 200 mV. The error in the event rate $R$ is calculated using $R/\sqrt{N}$, where $N$ is the number of observed blockade events. We plot the extracted event rate values as a function of voltage in Figure 8.5(c).

As the voltage is increased, the event rate initially increases exponentially, but at voltages larger than 150 mV it attains a constant value. We fit an exponential function to the data for voltages $\leq 150$ mV, as shown by the grey line in Fig. 8.5(c), and find an exponent of $0.048 \pm 0.002 \text{ mV}^{-1}$.

The exponential rate dependence can simply be described with a model where molecules cross a potential barrier $U'$ under influence of the electrical potential $\Delta U = nze\Delta V$. For the expected rate we can write $R \propto \exp((U' - \Delta U)/k_B T)$, with $n$ the number of charged monomers units of effective charge $ze$ within the potential drop $\Delta V$ [26]. Using these equations we find $nz \approx 1.2$. This value underestimates the real value because the actual potential drop involved in crossing the barrier is smaller than the applied potential $V$. The measured barrier for RecA-coated dsDNA entry into the fabricated nanopore might result from the large persistence length of the the filaments. An exponential dependence in event rate as a function of voltage was previously measured for DNA translocation through the protein pore $\alpha$-hemolysin [25–27], and recently for protein (BSA) translocation through a synthetic nanopore [28]. The event rate of DNA translocation was measured to be linear in fabricated nanopores over voltage from 200 to 1000 mV [29, 30].

At voltages $> 150$ mV, the event rate does not further increase but attains a constant value of $13.4 \pm 0.6 \text{ s}^{-1}$, as shown by the grey line in Fig. 8.5(c). A cross-over at higher voltages from an exponential to a roughly linear regime, was previously reported for the $\alpha$-hemolysin biological nanopore [25, 27], comparing fairly well to theoretical predictions [31, 32]. The measured constant event rate might reflect an oppressed rate due to polymer-polymer interactions, diffusion-limited transport, and/or modified electric field distributions inside the nanopore due to the high event rate.

### 8.6 Discussion and conclusions

We measured translocation of fully-recA coated dsDNA through solid-state nanopores. The translocation events of the nucleoprotein filaments show a large spread in event time duration as well as in the conductance blockade. We separate the events in two distinct groups, based on their conductance-blockade value. We find that the event duration of the high-conductance-blockade events
The event rate becomes larger as the voltage is increased. (b) Probability distribution to observe no RecA-coated dsDNA blockade events, \( P_0 \), for a elapsed time, \( t \), at 120, 130, 140, 160, and 200 mV. The solid lines are exponential fits to the data at each voltage value, showing good comparison. (c) The event rate of RecA-coated dsDNA conductance blockades as a function of voltage. Each value is extracted from an exponential fit such as the ones shown in (b). The event rate exponentially increases at low voltages \( \leq 150 \) mV, and attains a constant value at high voltages \( > 150 \) mV. The two regimes are fitted by an exponential and a constant, respectively, as shown by the two grey lines.

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data at 120 mV in Fig. 8.4(b) and at 180 mV in Fig. 8.3(b)) This may indicate an interaction of the protein with the sidewalls of the nanopore which hinders free translocation. Chemical coating of nanopores might result in a reduced spread in the event duration and hence yield a better identification of the translocation events [33].

We find that the event rate of RecA-coated dsDNA molecules can be well described by a Poisson process. We measure the event rate as a function of the applied voltage and identify two different regimes. In the low-voltage regime, the event rate is found to increases exponentially with voltage, whereas in the high-voltage regime it attains a constant value. Measurements of the event rate versus voltage on bare dsDNA and RecA-coated dsDNA filaments at different concentrations are expected to yield more information on the occurrence of the constant value and cross-over (at 150 mV) between the two regimes.

In conclusion, we are able to fully coat dsDNA molecules with the protein RecA to yield nucleoprotein filaments with a larger diameter and persistence length than bare dsDNA. We perform RecA-coated dsDNA translocation experiments and measure blockade events with a large variation in time duration and conductance blockade. The time duration of the high-conductance-blockade events depends on the length of the nucleoprotein filament, and their conductance blockade compares well to the structural information available. The event rate versus voltage of the nucleoprotein filaments shows an exponential increase at low voltages and a cross-over to a constant value at 150 mV. The reported translocation experiments are the first translocations of DNA-protein structures. The detection of RecA-dsDNA filaments shows great promise to provide for an efficient way to identify sequences on a target DNA strand using nanopores.

8.7 Acknowledgments

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References


Summary

This thesis describes experimental work on a novel type of devices that are capable of detecting single-(bio)molecules, namely nanometer-sized pores, or nanopores. The detection scheme is in principle very simple. Individual nanopores are placed in between two electrolyte-filled liquid compartments, forming the only connection between them. Electrodes are positioned on either side of the nanopore and are used to apply electrical fields and measure ionic currents. Adding negatively charged DNA to the negatively biased compartment electrophoretically drives molecules through the nanopore. During passage of a single DNA molecule the ionic current is temporarily blocked, as part of the liquid volume carrying the current is now occupied by the DNA. In this thesis, we report on the fabrication and characterization of solid-state nanopores, and their use in single-molecule translocation studies.

We start with a general introduction discussing the broader research fields of biophysics, nanotechnology and nanobiotechnology, before turning to nanopores. We introduce the field of biophysics by giving examples of two classic biophysical research areas, and we briefly characterize nanotechnology by discussing its origin and basic technologies, and by giving examples of current achievements. The field of biophysics and nanotechnology meet in a research area called nanobiotechnology, and we highlight some specifically interesting studies. We then focus on nanopore research and describe the development of the coulter counter and present an overview of experimental results obtained using the α-haemolysin protein pore. We discuss the recently developed solid-state nanopores and conclude that they are promising single-molecule sensors.

Chapter 2 describes our endeavors to fabricate solid-state nanopores using a high-intensity electron beam in a transmission electron microscope. Focussing the beam on thin free-standing membranes creates nanopores of controlled sizes, down to 0.4 nm radius. The nanopore size and shape are characterized using
high-resolution transmission electron microscopy and electron-energy-loss two-
dimensional maps. Additionally, we show that these nanopores can also serve as a
template for the fabrication of nanoelectrodes. We examine these electrodes using
atomic and electrostatic force microscopy, and we demonstrate their applicability
in electrochemistry.

In addition to the demonstrated control over the lateral shape and size of
electron beam fabricated nanopores, we examine their three-dimensional geometry
and local surface composition in greater detail in Chapter 3. We identify a
material-dependent response to the electron beam from thickness-variation mea-
surements around nanopores fabricated in pure SiN and SiO$_2$/SiN/SiO$_2$ mem-
branes. Nanopores fabricated in pure SiN membranes show much smaller de-
pletion areas compared to nanopores fabricated in SiO$_2$ containing membranes.
Nanopore formation in the latter membranes also results in the formation of
$\approx 3$ nm-sized Si-rich particles. Furthermore, we study the use of electron beams
of different intensities on initially fabricated small nanopores in SiO$_2$ containing
membranes. We demonstrate that we can influence the sidewall angle and the lo-
cal material composition near the nanopore using either a de-focussed or a highly
focussed electron beam.

We experimentally characterize the fabricated solid-state nanopores in Chap-
ter 4 by studying ionic current fluctuations over a wide frequency range. In the
high-frequency regime ($f \gtrsim 1$ kHz), we find an increase in current power spectral
density with frequency, which we successfully model by a calculation of the John-
son noise. At low frequencies ($f \lesssim 100$ Hz) we observe $1/f$-type of noise, with
strong pore-to-pore variations. Here, we do not consider nanopores that show ex-
cessive low-frequency noise. We analyze the low-frequency noise of the remaining
nanopores at different salt concentrations and find that the noise power remark-
ably scales linearly with the inverse number of charge carriers, in agreement with
Hooge’s relation. We find a Hooge parameter $\alpha = (1.1 \pm 0.1) \times 10^{-4}$. We use the
obtained description of the noise over the entire frequency range to compute the
signal-to-noise ratio for DNA translocation. Varying the salt concentration and
nanopore diameter, we provide the parameters for optimal detection efficiency.

Despite the agreement between Hooge’s relation and the measured $1/f$ noise,
we also consider an alternative model in Chapter 5: ionic current fluctuations
originating from surface-charge fluctuations in nanopores. We compare the two
models and show that, whereas the models are not easily distinguished in the
low-salt regime, Hooge’s relation gives the best description for the low-frequency
noise in solid-state nanopores over the entire salt regime from $10^{-3}$ to 1.6 M KCl.

We complete the characterization of the fabricated solid-state nanopores in
Chapter 6 by examining their excessive low-frequency noise. We perform conduc-
tance and noise studies by scanning nanopores through the focus of an infrared laser beam and recording the ionic current through them simultaneously. The resulting conductance profiles show strong variations in both the magnitude of the conductance and in the low-frequency noise when a single nanopore is measured multiple times. Differences up to 5 orders of magnitude are found in the current power spectral density. We show a correlation between a decreased nanopore conductance and increased amount of ionic current fluctuations. In addition, we measure an unexpected double-peak ionic conductance profile. A simple model of a cylindrical nanopore that contains a nanometer-sized gaseous bubble (nanobubble) can explain the measured profile and account for the observed variations in the magnitude of the conductance. From these results, we infer that nanobubbles may act as a source of excessive low-frequency noise in solid-state nanopores.

We then use the fabricated and characterized nanopores in Chapter 7 to measure ion and double strand (ds)DNA transport at different salt concentrations. The ionic conductance shows a three-order-of-magnitude decrease with decreasing salt concentrations from 1 M to 1 µM, strongly deviating from bulk linear behavior. We successfully model the data by accounting for a salt-dependent surface charge of the nanopore. Translocation of 16.5 µm long dsDNA in salt concentrations ranging from 50 mM to 1 M is shown to result in either a decrease ([KCl] > 0.4 M) or increase of the ionic current ([KCl] < 0.4 M). The data are described by a model where current decreases result from the partial blocking of the pore due to the dsDNA area, and current increases are attributed to the motion of the counter ions screening the DNA backbone charge. We demonstrate that the two competing effects cancel at a KCl concentration of 370 ± 40 mM. Using this model, we infer an effective dsDNA charge of 0.58 ± 0.02 electron charges per basepair, equal to a 71 ± 2% charge reduction of the bare dsDNA charge.

Finally, in Chapter 8 we study the translocation of fully protein-coated ds-DNA molecules through solid-state nanopores. We use the recombination protein A (recA) to form stable nucleoprotein filament on the DNA, and we confirm their full coverage by atomic force microscopy (AFM). Translocation measurements on the nucleoprotein filaments result in large current blockade events, as compared to bare dsDNA, with a wide variety in time duration (≈ 10^{-4} – 10^{-1} s) and conductance blockade values (2.5 – 14 nS). The events can be classified in two distinct groups, depending on the value of the conductance blockade. From measurements on different nucleoprotein filament lengths we identify the events with high conductance blockades (11.9 ± 0.5 nS) with the translocation of dsDNA coated with recA. From a comparison of these conductance blockades values to measurements on bare dsDNA, we estimate a 8.5 nm diameter for the nucleoprotein filament, in good agreement with the available structural data. Furthermore, we also present
the rate of RecA-coated dsDNA current blockade events as a function of voltage, and find an exponential dependence at low voltages and a constant rate at high voltages. The two regimes are found cross over at 150 mV.

In conclusion, we have used tools from nanotechnology to fabricate solid-state nanopores. These nanometer-sized pores are characterized by studying ionic current fluctuations. We use the nanopores experimentally to detect and measure the transport of ions, DNA and fully protein-coated DNA molecules. The obtained results contribute to the development, understanding and use of the recently introduced solid-state nanopores as single-molecule (bio)sensors.

Ralph Smeets
May 2008
Samenvatting

Dit proefschrift beschrijft experimenteel werk aan nieuwe sensoren waarmee enkele (bio)moleculen gedetecteerd kunnen worden: gaatjes op nanometerschaal of nanogaatjes. De detectie berust op eenvoudige principes. Individuele nanogaatjes worden als enige connectie tussen twee elektrolyt gevulde vloeistofcompartmenten geplaatst. Elektrodes worden aan beide zijden van het nanogaatje gepositioneerd, en gebruikt om elektrische veldens aan te leggen en ionenstromen te meten. Het toevoegen van negatief geladen DNA aan het negatief geschakelde compartiment drijft moleculen elektroforetisch door het nanogaatje. De ionenstroom wordt tijdelijk geblokkeerd tijdens de passage van een enkel DNA molecuul, omdat een deel van het vloeistofvolume, verantwoordelijk voor de stroom, nu bezet wordt door het DNA. In dit proefschrift beschrijven wij de fabricage en karakterisering van nanogaatjes in vaste stof membranen, en bespreken we hun gebruik in enkel-moleculaire translocatie studies.

We beginnen met een algemene inleiding en behandelen eerst de brede onderzoeksvelden van de biofysica, nanotechnologie en nanobiotechnologie, alvorens we de nanogaatjes bespreken. We introduceren de biofysica door voorbeelden van twee klassieke biofysische onderzoeksbereiken te geven, en we karakteriseren de nanotechnologie door het begin, de basistechnologieën en voorbeelden van recente resultaten te bespreken. De onderzoeksgebieden van de biofysica en de nanotechnologie ontmoeten elkaar in een onderzoeksveld aangeduid als nanobiotechnologie, en wij belichten een aantal spectaculaire onderzoeken. Daarna richten we ons op het onderzoek aan nanogaatjes. We beschrijven de ontwikkeling van de coulter sensor en vermelden de resultaten behaald met het gebruik van het α-hemolysin eiwitgaatje. Vervolgens bespreken we de recent ontwikkelde nanometerschaal gaatjes in vaste stof membranen en concluderen dat dit veelbelovende enkel-moleculaire sensoren zijn.

In Hoofdstuk 2 beschrijven we de fabricage van nanogaatjes in vaste stof
Samenvatting

membranen door het gebruik van een hoge intensiteit elektronenbundel in een transmissie elektronenmicroscoop. Het focuseren van de bundel op een dun, vrijstaand membraan resulteert in gaatjes op de nanometerschaal, met een minimale straal van 0.4 nm. De grootte en vorm van de nanogaatjes zijn gekarakteriseerd door middel van hoge-resolutie elektronenmicroscopie en tweedimensionale afbeeldingen van het elektron-energieverlies. Daarnaast tonen we aan dat de nanogaatjes ook gebruikt kunnen worden als mal voor de fabricage van nanometerschaal-elektroden. We gebruiken atomaire en elektrostatische krachtmicroscopie om deze elektroden te onderzoeken en we demonstreren hun elektrochemische toepasbaarheid.

Naast de vertoonde controle over de laterale vorm en grootte van de nanogaatjes, gefabriceerd met de elektronenbundel, onderzoeken we in Hoofdstuk 3 meer gedetailleerd hun driedimensionale geometrie en lokale oppervlakte-eigenschappen. We identificeren een materiaalafhankelijke reactie op de elektronenbundel door metingen van membraandikte variaties rond nanogaatjes gefabriceerd in zowel puur SiN als SiO$_2$/SiN/SiO$_2$ membranen. De nanogaatjes in puur SiN membranen bezitten veel kleinere gebieden van verdunning in vergelijking met nanogaatjes in SiO$_2$ bevattende membranen. Het fabriceren van gaatjes op nanometerschaal in de laatstgenoemde membranen resulteert ook in de vorming van ≈3 nm grote Si-rijke deeltjes. Verder hebben we het gebruik van elektronenbundels van verschillende intensiteit op initieel gefabriceerde kleine nanogaatjes in SiO$_2$ bevattende membranen bestudeerd. We demonstreren dat we de hoek van de zijwand en de lokale materiaalcompositie rondom het nanogaatje kunnen beïnvloeden door gebruik te maken van ofwel een niet-gefocusseerde ofwel een hoog-gefocusseerde elektronenbundel.

De nanometerschaal-gaatjes worden experimenteel gekarakteriseerd in Hoofdstuk 4 door de ionenstroomfluctuaties over een breed frequentiegebied te bestuderen. In het hoogfrequente gebied ($f \gtrsim 1$ kHz) vinden wij een toenemende stroom spectrale dichtheid met toenemende frequentie, in overeenstemming met de door ons berekende Johnson ruis. Bij lage frequenties ($f \lesssim 100$ Hz) observeren we $1/f$-type ruis, met sterke variaties tussen de nanogaatjes. In dit hoofdstuk blijven de nanogaatjes met excessieve laagfrequentie ruis buiten beschouwing. We analyseren de laagfrequente ruis van de overige nanogaatjes bij verschillende zoutconcentraties en we doen de verassende ontdekking dat het ruisvermogen lineair schaalt met het inverse aantal ladingsdragers, in overeenstemming met Hooge’s regel. We vinden een Hooge parameter $\alpha = (1.1 \pm 0.1) \times 10^{-4}$. We gebruiken de gevonden beschrijvingen van de ruis om de signaalruisverhouding voor DNA translocatie over het gehele frequentiegebied uit te rekenen. Door de zoutconcentratie en grootte van het nanogaatje te variëren, verkrijgen we de
parameters voor een optimale detectie efficiëntie.

Ondanks de goede overeenkomst tussen Hooge’s regel en de gemeten $1/f$ ruis, beschouwen we ook een alternatief model in Hoofdstuk 5. Het nieuwe model schrijft de ionenstroomfluctuaties toe aan oppervlakkeladingfluctuaties in de nanometerschaal-gaatjes. We vergelijken de twee modellen en tonen aan dat, hoewel ze niet gemakkelijk te onderscheiden zijn in het lage zoutgebied, Hooge’s relatie de beste beschrijving geeft van de laagfrequente ruis over het gehele zoutgebied van $10^{-3}$ tot 1.6 M KCl.

We besluiten de karakterisering van de gefabriceerde nanometerschaal-gaatjes in Hoofdstuk 6 door hun excessieve laagfrequente ruis te onderzoeken. We bestuderen de geleiding en ruis door tegelijkertijd nanogaatjes in een gefocussederde infrarode laserstraal te bewegen en de ionenstroom te meten. De resulterende geleidingsprofielen van veelvoudige metingen aan individuele nanogaatjes laten sterke variaties in zowel de grootte van de geleiding als de laagfrequente ruis zien. We meten verschillen tot wel 5 ordes van grootte in de stroom spectrale dichtheid. We laten zien dat een afname in geleiding en een toename in ionenstroomfluctuaties corruleren voor onze nanometerschaal-gaatjes. Daarnaast meten we een onverwacht dubbel gepiekt geleidingsprofiel. Een simpel model van een cilindrisch nanogaatje dat een nanometerschaal-gasbel (nanobel) bevat, verklaart zowel het gemeten profiel als de gemeten variaties in de grootte van de geleiding. Uit deze resultaten leiden we af dat nanobellen als een bron van excessieve laagfrequente ruis in nanogaatjes kunnen fungeren.

We gebruiken de gefabriceerde en gekarakteriseerde nanometerschaal-gaatjes in Hoofdstuk 7 om ionen en dubbelstrengs (ds)DNA transport bij verschillende zoutconcentraties te meten. We vinden slechts een drie orde van grootte afname in de ionen geleiding bij een verlaging van de zoutconcentratie van 1 M tot 1 $\mu$M. De geringe geleidingsafname wijkt sterk af van lineaire bulk geleiding. We bieden een goede beschrijving van de data door een zout afhankelijke oppervlakkelading van het nanogaatje te verdisconteren. Translocatie van 16.5 $\mu$m lang dsDNA in zoutconcentraties van 50 mM tot 1 M resulteert in ofwel een afname ($[\text{KCl}] > 0.4$ M) ofwel een toename ($[\text{KCl}] < 0.4$ M) van de ionenstroom. We beschrijven de data met een model waarin de stroomafname wordt toegeschreven aan een partiële blokkade van het nanogaatje door het DNA, en de stroomtoename het gevolg is van de beweging van de ionen die de lading van het DNA afschermen. We demonstreren dat de twee concurrente effecten elkaar opheffen bij een KCl concentratie van $370 \pm 40$ mM. Door gebruik te maken van dit model, vinden we een effectieve lading van $0.58 \pm 0.02$ elementaire ladingen per basenpaar voor het dsDNA, ofwel een $71 \pm 2\%$ ladingsreductie ten opzichte van de naakte DNA lading.
Ten slotte bestuderen we in Hoofdstuk 8 de translocatie van volledig met eiwit bedekte dsDNA moleculen door nanogaatjes. We gebruiken het recombinatie eiwit A (recA) om stabiele eiwitfilamenten op het DNA te vormen en we bevestigen de volledige bedekking met de atomaire krachtmicroscoop (AFM). Translocaties van het met eiwit bedekte DNA resulteren in tijdelijke grote stroomblokkades, vergeleken met het onbedekte DNA, met een grote variatie in tijdsduur ($\approx 10^{-4} - 10^{-1}$ s) en geleidingsblokkade ($2.5 - 14$ nS). De tijdelijke stroomblokkades kunnen in twee afzonderlijke groepen worden ingedeeld, gebaseerd op de waarde van de geleidingsblokkade. Door metingen aan DNA bedekt met eiwitfilamenten van verschillende lengtes, identificeren we de tijdelijke stroomblokkades met grote waardes van de geleidingsblokkade ($11.9 \pm 0.5$ nS) met de translocatie van het met eiwit bedekte dsDNA. Uit een vergelijking van deze metingen met de gemeten geleidingsblokkade voor onbedekt DNA, schatten we de diameter van het eiwit-DNA filament op 8.5 nm. Dit verhoudt zich goed tot de beschikbare structurele data. Vervolgens presenteren we ook het aantal stroomblokkades per seconde als functie van het voltage van het RecA-bedekte dsDNA. We vinden een exponentiële afhankelijkheid bij lage voltages en een constant aantal blokkades per seconde bij hoge voltages. De overgang tussen deze twee gebieden vindt bij 150 mV plaats.

Concluderend kunnen we stellen dat we gebruik gemaakt hebben van het gereedschap van de nanotechnologie om nanometerschaal-gaatjes in vaste stof membranen te fabriceren. Deze nanogaatjes zijn gekarakteriseerd door het bestuderen van ionenstroomfluctuaties. We gebruiken de nanogaatjes in experimenten om transport van ionen, DNA en volledig eiwit-bedekte DNA moleculen te meten. De verkregen resultaten dragen bij aan de ontwikkeling en het gebruik van de recent geïntroduceerde nanometerschaal-gaatjes in vaste stof membranen als enkel moleculaire (bio)sensoren.

Ralph Smeets
Mei 2008
In August 2003 I finished my education and obtained a M.Sc. degree in applied physics. My studies were finalized with a research project exploring the possibilities to use molecular motors in nanofabricated structures. During this project I learned to fabricate small-scale structures in the cleanroom, to use a range of microscopy techniques, and to carefully measure and critically analyze the obtained results. Moreover, I experienced that studying physics from a book is different from actually doing physics in a laboratory. The use of new techniques to study single biomolecules and the possibility to contribute to the understanding of processes inside cells appealed to me. I decided to learn more about this exciting field of research and started my Ph. D. in September 2003.

This chapter is dedicated to all the people that have contributed to the work, either directly by contributions to the experiments and scientific discussions, and/or indirectly by supporting me during the setbacks encountered. I am aware that this chapter is probably the most popular part of the thesis. Therefore, I will try to deviate a little from the ‘award winning speech style’, as often encountered on tv.

First, I would like to thank my colleagues of the molecular biophysics research group in Delft. I want to highlight their strengths, from which I learned a lot, by using the analogy of a football team. Naturally we play according to the offensive 4-3-3 strategy. Cees is the senior head coach of the team, who is also partly responsible for the sponsor deals and the hiring of new players. Although acquiring money is a continuous effort, with ‘Spinoza’ and ‘Kavli’ on our shirts you have secured a comfortable position to build the team. You look for talent that is passionate about the game, and with great enthusiasm you push the team to reach a superior position on the ranking table. Communication skills, necessary to show the game to the outside world, are highly emphasized, and you demand players to take full responsibility and accountability. Moreover, you provide room for great team-building outings and you are a stranger to strict
hierarchy; good suggestions on tactics are always welcome and 'Mr. Head Coach' is simply Cees. Nynke also coaches the team and recently achieved a head coach position herself, congratulations on a job well done! You create the atmosphere to make players drink, dream, and dine football by organizing meetings with world-class players, and offering specialized training abroad. You have a good eye for necessities and valuable details and you emphasize a systematic approach. Serge, is the tactics coach of the team, passionate to spend hours in front of the white board to discuss player movements, to analyze the essential elements behind the final score, and to determine future strategies for improved players. Suzanne is the team’s goalkeeper, and forms a loyal and trustworthy backbone of the line of defense. She corrects the team’s mistakes and prevents us from getting one goal down. Meng-Yue, the magician from China, is the left full back. She masters the speciality of excelling in small spaces during the game. Diego is the team’s sweeper, or center-back. With his Argentian winner mentality present within every vein, he will most certainly eliminate every possible opponent’s attack. Marcel is the right full back, displaying great loyalty to his defending tasks, and adding his open and warm personality to the team. Martin is the second center-back who also acts as the defensive midfielder. He is renowned for the ability to defend his territory and is not afraid to use the handbreak or a bicycle kick, when necessary. Moreover, he is at the heart of most successful attacks. Koen is the left wide midfielder with incredible drive and stamina, and very capable to argue and confuse the referee to turn decisions to our advantage. Frank is the right wide midfielder, untraceable for opponents and combines hard work with a loose attitude. Daniel is positioned as the left winger. He has the ability to read the game and moves either inside-to-outside or outside-to-inside to create his own space on the pitch. Ulrich is the naturally born number ‘10’, the attacking midfielder. He has the ability to score and to make other people score. He strives for excelling goals and passes, and technical perfection is at the heart of his game. His knowledge, guidance, inspiration and helpful attitude have been invaluable to my research. Thijn is the right winger, capable of stunning the audience with incalculable moves. He also provides great support to the team by perfect deliveries into the box. Iddo is the team’s striker, combining a nearly perfect efficiency with a lack of egoism.

The drawback of the football team analogy is the limited number of players on the pitch. Many more players do deserve an appearance into the team. Here, I would like to thank people affected by the curse of the blessed nanopores. Arnold, my predecessor, for your well-considered working methods, and Stefan, my continuator, for your appreciated contributions and novel ideas. Kees, Kiona, and Georgia for your persistence and pleasure of cooperation during your final
research projects. Michiel, for the ease of working together and your involvement, and Ya-Hui, Serge, and Peter for making the impossible DNA possible. Adam and Gary for your stimulating energy, and Bernard, Stijn, Onno, Derek, Nadine, and Christine for tips and discussions.

Apart from the people involved in nanopore research, I would like to especially thank some other (former) group members. Anja, Emmylou, Elsemarieke, and Liset for their secretarial support and relativizing lively attitude. Jaap, Jelle, Jack, Dick, and Jacob for their technical support, and Jan and Iwijn for their company during aula dinners. Irene for her responsibility, and Bernhard, Igor, Andrea, Fernando, and Ralf for their jokes and fun characters. Also, the more recently joined Ph. D. candidates Marijn and Zhuangxiong. Marijn, for your sharp, clever attitude, and Zhuangxiong for your fast personal growth and for conversations on cultural differences. Emile van der Drift, Marco van der Krogt, Anja van Langen-Suurling, Raoul Mattern, Arnold van Run and Marc Zuiddam of the DIMES nanofacility for their continuous devotion and suggestions. I would also like to thank everyone, not specifically mentioned, who contributed to my research and all the members of the molecular biophysics group.

Before starting the Ph. D. research my interests were shaped and influenced by a number of people. First, I would like to thank my secondary school teachers. Humblet, for his reassuring trust, Rutten, for his inquisitive mind, Nelissen, for his university level mathematics classes and warnings of the temptations of Delft student life, and deLey, for his insights into physics. Subsequently, I would like to thank Michel van Putten, for his serene guidance during my final B. Sc. research project, John Howard for his relaxed and kind supervision of my internship, and Stefan Diez for collaboration and insights on my final M. Sc. research project. Finally, I would like to acknowledge Keith and Jeong-O, for their help and friendly attitude stimulating my interest in research.

Support of all my friends an family was crucial to be able to successfully finish my Ph. D. The research is inevitably accompanied by setbacks and the patience of people surrounding you is tested by occasional long working hours. Here, I would like to thank all my friends and family for their honest and warm interest. I want to specifically mention Jan-Willem and Rik for being my paranimfen and socially involved friends. Wendy and Michiel, for their understanding and continuous support. My parents, Els and Wim, for their financial and emotional help enabling me to study. ‘Oma’ for being a great grandparent. Chantal for your love, strength, advice, focus on true values, relaxing evenings, and your extra activities necessary due to my absence.

Ralph Smeets
May 2008
Curriculum Vitae

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02-05-1977  Born in Geleen, the Netherlands.

1989-1995  Secondary school (V.W.O.),
            Scholengemeenschap Groenewald in Stein.

1995-2003  M.Sc. Applied Physics (with honors),
            Delft University of Technology.

1998-1999  Student council representative,
            Delft University of Technology.

2000       Research project at VP Instruments B.V.
            Delft, the Netherlands.

2001       Internship at the Australian National University,
            Canberra, Australia.

2003-2008  Ph.D. research at Delft University of Technology,
            supervised by prof. dr. N.H. Dekker and prof. dr. C. Dekker
List of Publications

1. Translocation of RecA-coated DNA molecules through solid-state nanopores
   In preparation.

2. Comparison of two models for the low-frequency noise in solid-state nanopores
   In preparation.

3. Control of shape and material composition of solid-state nanopores
   M.Y. Wu, R.M.M. Smeets, M. Zandbergen, D. Krapf, P.E. Batson, N.H. Dekker,
   C. Dekker, and H.W. Zandbergen.
   Submitted to Nano Letters.

4. Noise in solid-state nanopores

5. Nanobubbles in solid-state nanopores

6. Direct force measurements on DNA in a solid-state nanopore
   U.F. Keyser, B.N. Koeleman, S. van Dorp, D. Krapf, R.M.M. Smeets, S.G. Lemay,
   N.H. Dekker, C. Dekker.

7. Fabrication and Characterization of Nanopore-Based Electrodes with Radii down to 2 nm
8. *Salt dependence of ion transport and DNA translocation through solid-state nanopores*

9. *Nanopore tomography of a laser focus*

10. *High Rectifying Efficiencies of Microtubule Motility on Kinesin-Coated Gold Nanostructures*

11. *Lithographically fabricated nanopore-based electrodes for electrochemistry*