Ion Track-Based Nanofluidic Biosensors

3

Yamili Toum Terrones, Vanina M. Cayón, Gregorio Laucirica, M. Lorena Cortez, María Eugenia Toimil-Molares, Christina Trautmann, Waldemar A. Marmisollé, and Omar Azzaroni

Abstract

During the past decade, solid-state nanopores and nanochannels (SSNs) have emerged as a new class of devices for the creation of nanofluidic platforms with diverse applications. In particular, the precise control of ion transport achieved by SSNs paved the way to the development of specific and efficient biological and chemical iontronic sensors with promising technological potential. As biological ion channels play crucial roles in the regulation of vital processes for human cells, they have been a huge source of inspiration toward the design and construction of more sophisticated SSN devices. Today, the academic research on the topic has evolved to many concrete and practical usages, reflecting the potential commercial value of SSNs. Among the different methods available for the nanofabrication of single SSNs, high-energy ion beam (~MeV–GeV) techniques coupled to etching chemical processes are one of the most used due to their control on the size and geometry of the pore. The combination of this advanced nanofabrication technology and different surface functionalization strategies to confer specific target moiety responsiveness to the SSNs were the key point for the extraordinary advances in the area. This chapter aims to provide a closer look

Y. Toum Terrones · V. M. Cayón · G. Laucirica · M. L. Cortez · W. A. Marmisollé · O. Azzaroni (✉)
Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), Departamento de Química, Facultad de Ciencias Exactas, Universidad Nacional de La Plata, La Plata, Argentina
e-mail: azzaroni@inifta.unlp.edu.ar

M. E. Toimil-Molares
GSI Helmholtzzentrum für Schwerionenforschung, Darmstadt, Germany

C. Trautmann
GSI Helmholtzzentrum für Schwerionenforschung, Darmstadt, Germany

Technische Universität Darmstadt, Materialwissenschaft, Darmstadt, Germany

P. Chandra, K. Mahato (eds.), *Miniaturized Biosensing Devices*,
https://doi.org/10.1007/978-981-16-9897-2_3

at the fabrication of SSNs by the ion-track-etching technology and the functionalization strategies in order to build SSNs for biosensing purposes.

Keywords

Nanofluidic · Sensors · Bioanalysis · Analytical performance

3.1 Introduction

Living systems have always been a limitless source of inspiration for scientists both in basic and applied investigations. In particular, ion channels are ubiquitous in nature and play vital roles in many important life processes, such as signal propagation and processing (Pérez-Mitta et al. 2017a; Zhang et al. 2018). Inspired by the key functions of biological ion channels, the development of fully-abiotic solid-state nanochannels (SSNs) has been at the forefront of materials science in recent years (Gleich et al. 2010). Thus, tailorable nanochannels having dimensions comparable to the size of biological molecules and mimicking the function of biological ion channels have attracted attention based on their use as exceptionally sensitive molecule biosensors (Ali et al. 2008). As the biosensing capabilities of the SSNs considerably depend on the surface characteristics of their inner walls, the finely tuning over the surface properties at the nanoscale level plays a crucial role in the biosensing performance.

Among all nanofabrication techniques, ion-track-etching technology is a well-established technique for the development of SSNs in polymer membranes (Spohr 1990). Track-etched foils, as biological protein nanopores, have interesting features such as their selective and asymmetric transport but with enhanced chemical stability, which make them excellent substrates for the design of complex (bio)sensing devices (Tagliazucchi and Szleifer 2016). The surface chemical groups of track-etched nanochannels serve as strategic sites for the proper anchoring of biological building blocks with the capability of binding to or reacting with specific analytes. The integration of these recognition elements into SSNs allows for sensing of biological analytes based on the ionic transport across the nanochannels.

In this chapter, we will first introduce the basic concepts of the ion track-etching technology for the nanofabrication of abiotic nanochannels and the principles that govern the transduction of ionic signals. Then, the strategies for the integration of bioreceptors and some concrete examples of biorecognition mechanisms are addressed.

3.2 Nanofabrication: Ion-Track-Etching Technology

Ion-track-etching technology has been widely used for the creation of nanochannels in both polymeric materials such as polyethylene terephthalate (PET), polyimide (PI), and polycarbonate (PC), as well as other inorganic materials such as silica and

mica (Toimil-Molares 2012; Trautmann 2009). It involves two main steps: (a) the generation of an ion-track via the irradiation of the material with swift heavy ions and (b) the selective ion-track dissolution by performing an appropriate chemical etching.

3.2.1 Swift Heavy-Ion Irradiation

In the first step, a polymeric foil is irradiated with swift heavy ions of MeV–GeV energy (Fig. 3.1(a)). Each swift heavy ion generates along its trajectory in the material a damaged region known as ion-track. For example, at the MAT facilities available at the UNILAC linear accelerator of the GSI Helmholtz Center for Heavy Ion Research (Darmstadt, Germany), materials can be irradiated with heavy ions (^{197}Au, ^{206}Pb, and ^{238}U) with energies of up to 11.4 MeV per nucleon (~15% velocity of the light). Moreover, the number of ions per area unity (ion fluence) that impacts the sample can be adjusted between a single ion and ~10^{11} ions/cm^2 (Fig. 3.1(a)) (Toimil-Molares 2012).

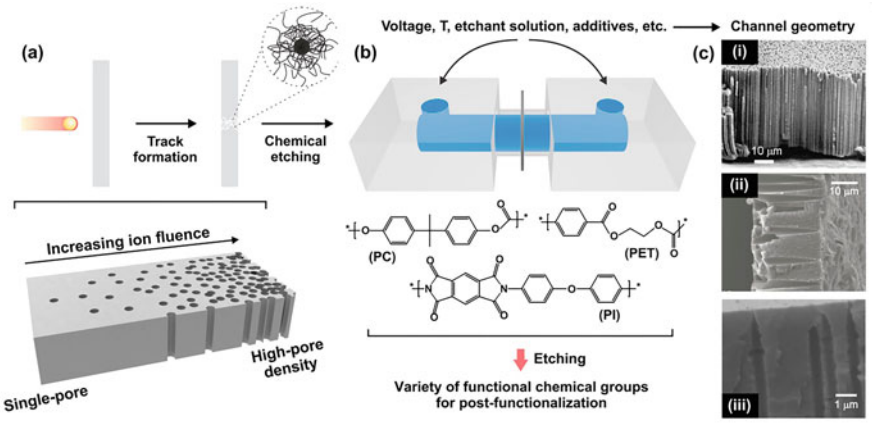

Fig. 3.1 Schematic illustration of ion-track-etching technology. (**a**) Foil irradiation with swift heavy ions (top), which allows the modulation of the channel density by tuning the ion fluence (bottom). (**b**) Chemical etching of the ion tracks by placing the irradiated polymer foil in between two compartments (top), which creates the nanochannels and generates reactive surface groups for post-functionalization depending on the identity of the polymer foil (PC, PET, or PI) (bottom). (**c**) SEM cross-section images of polymer membranes with (1) cylindrical pores (PI) adapted with permissions from (Trautmann et al. 1996). Copyright © 1996 Published by Elsevier B.V.; (2) conical channels (PC) adapted with permissions from (Pérez-Mitta et al. 2018a). Copyright © 2018 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.; (3) bullet-shaped channels (PET) adapted with permissions from (Laucirica et al. 2020c). Copyright © 2020 Published by Elsevier B.V

3.2.2 Chemical Etching

Taking advantage of the physicochemical differences of the track region compared to the bulk material, it is possible to transform every ion-track into a nanochannel by exposing the irradiated membrane to an adequate chemical (etchant agent) that reacts and selectively dissolves the cylindrical track region (Fig. 3.1(b)). This anisotropic dissolution rate along the damaged ion-track and the isotropic dissolution rate of the bulk undamaged polymer material are denoted track etching rate (v_t) and bulk etching rate (v_b), respectively (Apel and Fink 2004). By modulating the relation between v_t and v_b (track-to-bulk etch ratio $v = v_t/v_b$), it is possible to generate channels with different geometries (Fig. 3.1(c)). On the one hand, with high v values, channels with cylindrical geometries (symmetrical channels) are obtained. For example, in the case of materials such as PC and PET, cylindrical channels can be attained by exposing the irradiated membrane to highly concentrated NaOH solutions (typically 2–6 M), whereas in the case of PI, the etchant agent is usually a NaOCl solution with 13% of active Cl at pH ~9. (Apel and Fink 2004; Pérez-Mitta et al. 2018a) On the other hand, a low v value with asymmetrical etching conditions leads to asymmetrical channels, such as conical or bullet-shaped channels. For example, conical channels can be created by exposing one side of the membrane to the etchant solution and the opposite side to a stopping solution that neutralizes the etchant. In the case of PI, this is achieved by employing a NaOCl solution with active Cl at pH ~12 and a reducing agent solution as an etchant and stopping solution, respectively. In the case of PC and PET, this is achieved by employing a highly concentrated NaOH solution and an acidic solution as an etchant and stopping solution, respectively (Pérez-Mitta et al. 2016, 2018a; Siwy et al. 2003). Bullet-shaped channels can be obtained in PET by adding a small portion of surfactant to the NaOH solution only on one side (Laucirica et al. 2019a, 2020a). All these etching protocols can be performed using different etching time, etchant concentration, and temperature, also in combination with, e.g., applying a transmembrane voltage, pre-exposing the foils with UV light, varying additive concentrations, etc. (Apel and Fink 2004; Apel et al. 2007) The appropriate selection of the specific etching conditions is crucial since it determines the final structural parameters such as the channel aperture sizes and geometry.

In summary, ion-track etching is a versatile and attractive technology to create nanochannels, especially in polymeric materials. The possibility to finely tune the channel density and the relatively straightforward experimental protocol required to adjust the geometrical channel parameters via the appropriate selection of etching conditions are the main advantages. Furthermore, the surface of the track-etched channels synthesized in polymeric materials can be functionalized, exposing a variety of functional chemical groups, such as carboxylic acids, alcohols, phenoxy groups, and amines, which can be easily derivatized for the subsequent integration of stimuli-responsive building blocks or recognition elements (Pérez-Mitta et al. 2015a, 2017b; Laucirica et al. 2020b).

3.3 Transduction by Iontronic Signals

Sensing with nanofluidic devices has been possible by two different approaches: resistive-pulse sensing and steady-state measurements. The former is usually performed by employing nanopores (low aspect ratio, i.e., length ~ radius) and recording the current as a function of time. In contrast, steady-state measurements are more often carried out in solid-state nanochannels (SSNs) (high aspect ratio or, i.e., length >> radius) such as polymer track-etched or anodic aluminum oxide membranes. In this chapter, we will be focusing only on sensing by steady-state measurements (Pérez-Mitta et al. 2017a).

Biosensing with SSNs by steady-state measurements generally involves the recording of current–voltage (*I–V*) curves in the absence and presence of the target analyte. For this, the polymeric membrane containing nanochannels is placed between two half-compartments of a conductivity cell, and a two or four-electrode arrangement is connected to a potentiostat or sourcemeter (with voltage source) to control the transmembrane voltage and to simultaneously record the current. Taking into account that transmembrane current is given by the ion flux, ions are considered the signal carriers and the signal response is often referred to as iontronic output.

To detect and quantify the presence of a target analyte, a measurable change in the iontronic output must be generated. For this, the analyte should trigger a change in the physicochemical properties of the nanochannel surface that affects the readout. Given this, one of the main approaches for constructing sensors and biosensors based on SSNs involves the integration of recognition elements on the nanochannel surface that selectively interact with the analyte in such a way that the channel undergoes a physicochemical change in the presence of the target molecule. There are three established mechanisms to control the iontronic readout via changes on the nanochannel surface: (a) modulation of the surface charge, (b) steric effects or volume exclusion, and (c) wettability changes (Pérez-Mitta et al. 2019). In the case of bio- and chemical sensors, usually, the transduction mechanisms are focused on the phenomena (a) and (b), which will be examined in this chapter. Additional details about ion transport in nanochannels and the different transduction mechanisms are available in Ref. (Tagliazucchi and Szleifer 2016).

3.3.1 Iontronic Response Modulated by Surface Charges

The immersion of a charged surface into an electrolyte solution promotes a redistribution of ions in the interface region of the solution. This region at the vicinity of the surface is designated as the electrical double layer (EDL) in honor of the first models proposed to describe its structure and behavior (Bard and Faulkner 2001; Brett and Oliveira Brett 1993). Within the EDL, the electrostatic potential differs from the value of the bulk solution and, as a result of electrostatic interaction between the ions and the surface charged groups, this region is characterized by an enrichment of counterions (ions with opposite charge polarity than the surface groups) and a depletion of co-ions (ions with the same charge polarity than the surface groups).

The characteristic thickness of the EDL is called the Debye length (λ_D), and its value only depends on parameters related to the electrolyte solution. Typically, λ_D decreases as the electrolyte concentration increases, for example, λ_D acquires values of 9.61 nm and 0.96 nm (at 298 K) for 1:1 aqueous electrolyte solutions with concentrations of 0.001 M and 0.1 M, respectively. In the case of nanochannels, when the channel radius (at least at its smallest aperture) is comparable to the λ_D, (Pérez-Mitta et al. 2017c) the ion concentration inside the channel is controlled by the surface charges, and, therefore, the ion transport across these nanostructures is selective to counterions. The result is the polarization of the ionic concentration (ICP) inside of the channel.

The symmetry of the channel plays a key role in the determination of the iontronic response. Symmetrical systems such as cylindrical channels act as ohmic resistors (Fig. 3.2(a)). Therefore, the response of these devices is characterized by a linear relationship between the current and the transmembrane voltage. Moreover, under surface charge-governed transport conditions, the conductance G (slope of I–V curve) of the channel is directly related to the surface charge density. This behavior was comprehensively explained in a seminal work reported by Schoch and Renaud (Schoch and Renaud 2005). These authors found that the conductance of negatively charged silica nanoslits obeyed the following relation:

$$G = 10^3 \cdot \left(\mu_{K+} + \mu_{Cl-}\right) \cdot C \cdot N_A \cdot e \cdot \frac{w \cdot h}{d} + 2 \cdot \mu_{K+} \cdot \sigma_s^* \cdot \frac{w}{d} \qquad (3.1)$$

where μ_i corresponds to the i-ion mobility, C is the electrolyte concentration, N_A is the Avogadro number, e is the electron charge, σ_s^* is the effective surface charge, and w, h, and d are the weight, height, and length of nanoslits, respectively. Under surface charge-governed transport, i.e., low salt concentration enabling to obtain a λ_D comparable to the slit size, G is dominated by the second term of the right, and therefore, the conductance is dependent on the effective surface charge. Even more, if there is a change in the surface charge, it would be evidenced by a variation in the conductance G. Therefore, one of the most used strategies facing up the development of (bio)sensing devices based on symmetrical track-etched nanochannels involves the modification of the nanostructures with recognition elements that in the presence of certain analytes promote changes in the surface charge density. Thus, the analyte concentration can be related to the changes of the channel conductance and, therefore, quantified in different samples (Fig. 3.2(a)).

When the channel symmetry is disrupted, the ion response is quite different. SSNs with broken symmetry present non-ohmic ion transport properties (Siwy 2006). The iontronic response in asymmetrically modified channels and geometrically asymmetric channels such as conical or bullet-shaped channels is characterized by an ionic current rectification regime (ICR) (Fig. 3.2(b)) (Cervera et al. 2006). In a rectifying regime, the conductance of the system depends on the transmembrane voltage polarity. For one polarization, the ionic current is high (high-conductance branch), but the ionic current is low for the opposite transmembrane polarization (low-conductance branch). This asymmetric behavior is sometimes referred to as

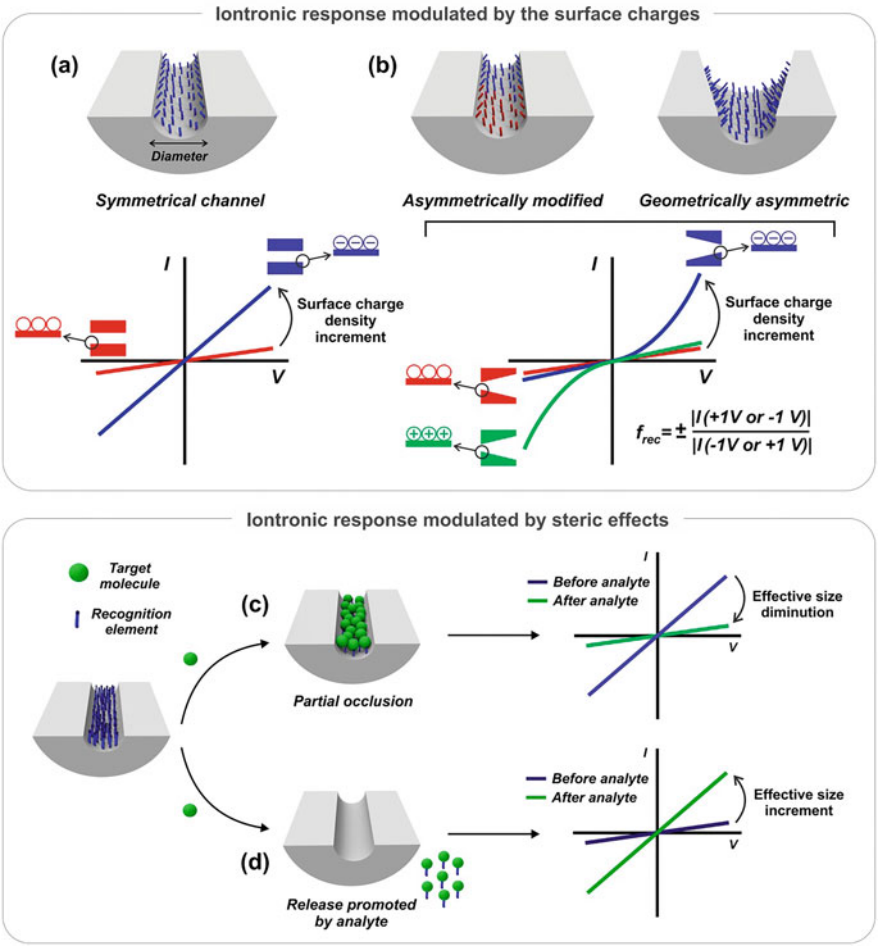

Fig. 3.2 Transduction mechanisms in SSNs. Surface charge effect: correlation between the iontronic response and the surface charge in (**a**) cylindrical (symmetrical) nanochannels and (**b**) asymmetrical nanochannels. Steric effect: changes in the iontronic output due to the partial occlusion (**c**) or aperture (**d**) promoted by the analyte

current rectification or diode-like behavior because of the analogy with electronic diodes. The type of surface charge (positive or negative) determines the direction of the rectification. Thus, the ICR is referred to as anion-driven (anion-selective) rectification or cation-driven (cation-selective) rectification for positively and negatively charged channels, respectively. Then, the diode-like features of the *I–V* curve allow inferring both the surface charge density and its sign (Fig. 3.2(b)). Moreover, the efficiency of the rectification can be quantified by using the rectification factor (f_{rec}) defined as the module of the ratio between the ion currents at the high- and low-conductance branches. Also, the sign of f_{rec} is determined by the sign of the

surface charge. Thus, f_{rec} is a parameter regulated by the surface charge magnitude (Pérez-Mitta et al. 2017b; Yameen et al. 2010). Hence, similarly to the symmetrical case, if the analyte promotes changes in the surface charge density, it could be evidenced by the variation in either the high-conductance branch current values or the rectification efficiency (f_{rec} values) (Fig. 3.2(b)).

3.3.2 Iontronic Response Modulated by Steric Effects

As shown in Eq. (3.1), the channel dimension is a determining magnitude of the channel conductance. Larger inner space enables a higher flux of ions yielding higher conductance values. In contrast, partial or complete blockage of the channel leads to a decrease in conductance (Yameen et al. 2009). These concepts provide the basis for (bio)sensing via steric effects, and at least two common strategies are known. On the one hand, it is possible to decorate the channel surface with a recognition element that interacts with a bulky analyte causing a partial channel blockage, which decreases the conductance (Fig. 3.2(c)) (Guo et al. 2019). On the other hand, if the recognition element is released away from the channel by the interaction with the target analyte, the inner space increases and, consequently, the conductance rises (Fig. 3.2(d)) (Chen et al. 2016; Liu et al. 2016). In both cases, the analyte concentration can be correlated with the changes in the channel conductance and, therefore, quantified in different samples.

3.4 Ion-Track -Based Nanofluidic Biosensors

A biosensor integrates two basic components in the same device: the bioreceptor—a biological molecule that recognizes the target analyte—and the transducer, which is capable of converting the recognition event into a measurable signal (Lee and Mutharasan 2005). The appropriate selection of the recognition elements is central to developing a sensor with the desired selectivity, sensibility, and robustness. Thus, surface functionalization with biological compounds such as DNA, RNA, enzymes, proteins, aptamers, and antibodies, among others, has become popular because these bio(macro)molecules provide (1) a specific recognition of target analytes, (2) high efficiency for specific coupled reactions, as it is the case of enzymes, and (3) the possibility of promoting a signal amplification by fusing biomolecular systems and nanotechnology (Ding et al. 2019). In this context, by combining the inherent signal amplification properties of track-etched nanochannels and the capability of biological macromolecules to bind/react with specific analytes, a variety of biosensors for a vast number of analytes (ions, nucleic acids, proteins, cells, drugs, amino acids, sugars, neurotransmitters, pollutants, and gases) has been developed (Pérez-Mitta et al. 2019). These devices exploit not only the different functionalization strategies of SSNs but also the different mechanisms of recognition between the bioreceptor and the target analytes. In the following sections, we will focus the attention on the general strategies for the integration of biorecognition

elements into track-etched SSNs, subsequently revising some paradigmatic examples of responsive systems based on interactions of the bioreceptor–target analyte for the different families of bioreceptors.

3.4.1 Strategies for the Integration of Bioreceptors

The modification strategy plays a key role in conferring specific properties to the inner surface of the nanochannels, such as wettability, surface charge, and functionality (responsiveness, catalytic activity, biological affinity), or in reducing undesired interactions. Chemical functionalization of nanochannels consists of one or more steps where, generally, the first step is a chemical reaction that allows the introduction of chemical groups onto the inner surface of the channels that serve to attach the molecules of interest in the following step. Here, some of the most employed functionalization strategies are reviewed and commented.

3.4.1.1 Covalent Functionalization: EDC/NHS Coupling Reaction

Carboxylate groups on the etched PET surface can be derivatized by producing amide linking groups from the reaction with primary amine groups in a variety of building blocks. The EDC/NHS approach is a very extended strategy for promoting this coupling reaction. N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDC) combined with N-hydroxysulfosuccinimide (NHS) or sulfo-NHS is employed for activating carboxylate groups for its subsequent reaction with nucleophiles (primary amine groups, for example) (Fig. 3.3(a)) (Apel et al. 2011). This method has proved to be successful, and it is commonly used in the construction of nanopore-based biosensors (Ali et al. 2010a; Vlassiouk et al. 2009).

3.4.1.2 Electrostatic Self-Assembly

Electrostatic adsorption of polyelectrolytes is an alternative technique to covalent chemistry that allows creating a thin polymer film on nanochannel surfaces (Fig. 3.3(b)). In a typical experiment, the nanochannel membrane is immersed into a solution of a polyelectrolyte with the opposite charge to that of the channel walls (Pérez-Mitta et al. 2017b; Laucirica et al. 2019b, 2020d). Although polyelectrolyte deposition on flat surfaces is a well-understood process (Netz and Andelman 2003), nanoconfinement effects have to be taken into account for adsorption in nanochannels. Therefore, a successful modification of its inner surfaces via electrostatic adsorption requires considering variables such as the channel diameter, the molecular weight of the polyelectrolyte, and the ionic strength and pH of the solution (Gilles et al. 2018).

After the first layer of electrolyte is absorbed on the surface, the net charge of the surface inverts and becomes the same as that of the adsorbed polyelectrolyte and is possible to sequentially adsorb another polyelectrolyte of opposite charge in order to assemble a polyelectrolyte multilayer. This process is known as layer-by-layer (LbL) self-assembly (Decher et al. 1992). Several groups have used the LbL self-assembly method to asymmetrically functionalize SSNs, observing that the rectifying

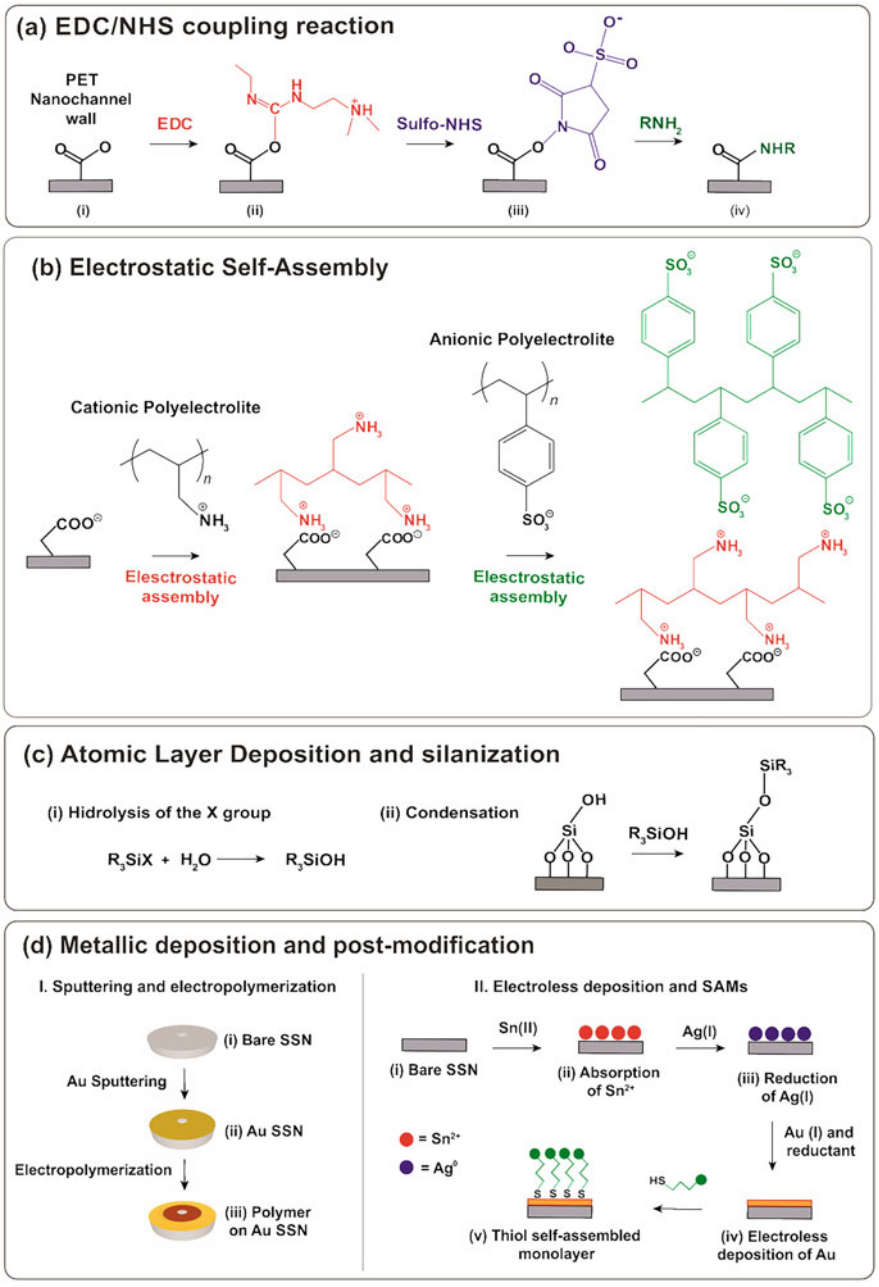

Fig. 3.3 Illustrative scheme of the different types of functionalization strategies: (**a**) EDC/NHS coupling reaction, (**b**) electrostatic self-assembly, (**c**) atomic layer deposition and silanization, and (**d**) metallic deposition and its possible postmodifications. (**b**) was adapted with permission (Ali et al. 2010c). Copyright © 2010 American Chemical Society

properties are strongly affected by the result of the conditions and the nature of the most external polyelectrolyte capping layer (Ali et al. 2010b; Ma et al. 2018).

3.4.1.3 Atomic Layer Deposition and Silanization

Another type of covalent method of surface functionalization of SSNs is the atomic layer deposition (ALD) process, with which thin films of a variety of materials, such as SiO_2, TiO_2, and Al_2O_3 oxides (Sander et al. 2004; George 2010; Spende et al. 2015; Sobel et al. 2015; Ruff et al. 2018; Kumari et al. 2018), can be produced. Silica surfaces present a combination of siloxane groups, which can behave as weak acids or weak bases depending on the pH conditions.

Moreover, organosilanes are commonly employed for silica functionalization. Silanes have general formulas $RSiX_3$, R_2SiX_2, or R_3SiX, where R is an organic group that confers functionality to the surface and X is a hydrolyzable group, which is lost during coupling to the silica surface. A representative scheme of this functionalization mechanism is presented in Fig. 3.3(c). This modification has proven to be successful in some systems, such as glass nanopores (Zhao et al. 2013) and alumina (Tan et al. 2011), although exploration in single-pore polymer nanochannels coated with SiO_2 continues to be a challenge.

3.4.1.4 Metallic Deposition and Postmodification

Nanochannel surfaces can also be metalized by thermal evaporation or *sputtering*, which allows the preparation of asymmetric gold coatings, i.e., in one side of the membrane (Pérez-Mitta et al. 2015b). The good conductivity of the Au film enables further functionalization steps of the metal surface by electropolymerization (Fig. 3.3 (d)) (Laucirica et al. 2020a; Pérez-Mitta et al. 2015c; Tian et al. 2010). The combination of both Au-sputtering and electropolymerization in nanochannels not only allows using the metalized membrane as an electrode (controlling its membrane potential and ionic transport) but also controlling the pore diameter.

Another efficient method to create metallic films is the electroless plating method (Ohno 1991). This technique is especially recommended for polymeric substrates that require mild modification conditions and can be described as an autocatalytic redox reaction in which metal cations in solution (Au(I), for example) are reduced onto a substrate to form a metal layer. Commonly, this process is carried out in the presence of surface-immobilized metal nanoparticles that act as catalysts of the reaction. As an example, the Au-electroless protocol employs a mixture of an Au (I) complex and a strong chemical reductant (e.g., formaldehyde) in the presence of Ag nanoparticles (Muench et al. 2011). The success of this methodology has been demonstrated by several authors on PET and PC track-etched membranes (Gao et al. 2014; De Leo et al. 2007).

On the other hand, self-assembled monolayers (SAMs) of thiols on gold (and other coinage metals) constitute a versatile pathway to introduce surface chemical functionalities (Love et al. 2005). Depending on the nature of the terminal group, the one-step self-assembly process can lead to the formation of a monolayer of predefined functional groups on the surface. This method has been commonly used for the functionalization of gold surfaces due to its ease of preparation and

the good stability of the gold–thiolate bond at ambient conditions (Fig. 3.3(d)). For the application of this surface chemistry to SSNs, the membranes must be previously metalized. In this sense, it is possible to coat the surfaces of nanochannels with a thin gold layer and then form a SAM of thiols with specific chemical functionalities (Tian et al. 2011; Martin et al. 2001; Nasir et al. 2014).

3.4.2 Biorecognition Mechanisms

The different biorecognition mechanisms are presented below according to the nature of the recognition element.

3.4.2.1 Nucleic Acids

In a seminal work, Harrel et al. presented in 2004 the integration of DNA into single conically shaped PC-based nanochannels coated with a thin layer of gold to build artificial ion channels (Harrell et al. 2004). The authors proved that the extent of rectification could be precisely controlled by either a simple chemical method (varying the DNA chain length) or a simple physical method (changing the nanochannel mouth diameter). After that, many efforts have been dedicated to the integration of DNA architectures into SSNs in order to achieve the detection of a wide variety of analytes including ions and nucleic acid molecules.

Mechanical Transduction Modulated by DNA Complex Structures

Buchsbaum et al. presented a new concept for building synthetic DNA-modified nanochannels that could simultaneously respond to pH and transmembrane potential changes (Buchsbaum et al. 2014). By attaching DNA oligomers containing protonable adenine (A) and cytosine (C) at the narrow opening of an asymmetric SSN, they showed that a pH-dependent reversible closing mechanism was responsible for the iontronic output. Specific DNA secondary structures known as G-quadruplexes (G4) were also employed for the construction of proton-gated nanofluidic nanochannels (Wang et al. 2009). After grafting the walls of a conical-shaped single track-etched nanochannel with G-rich human telomere strand (a type of G4 DNA), the DNA underwent a potassium-responsive conformational change between a random single-stranded structure (no K^+) and a four-stranded G4 structure (presence of K^+) (Fig. 3.4(a)), which induced a change in the effective pore size and consequently in the ion transport properties of the device (Fig. 3.4(b)). Furthermore, immobilizing C-quadruplex and G-quadruplex DNA molecules onto the top and bottom tip side of a cigar-shaped nanochannel, Liu et al. presented a bioinspired pH and K^+ double-gated nanosystem (Fig. 3.4(c)) (Liu et al. 2015). By adjusting K^+ and H^+ (chemical effectors) concentrations, the two gates of the nanochannel can be opened and closed alternately or simultaneously.

The Hybridization Strategy as Recognition and Binding Mechanism

Hybridization is the process by which a synthetic DNA probe or primer binds via Watson–Crick base pairing to a biological DNA target sequence (Khodakov et al.

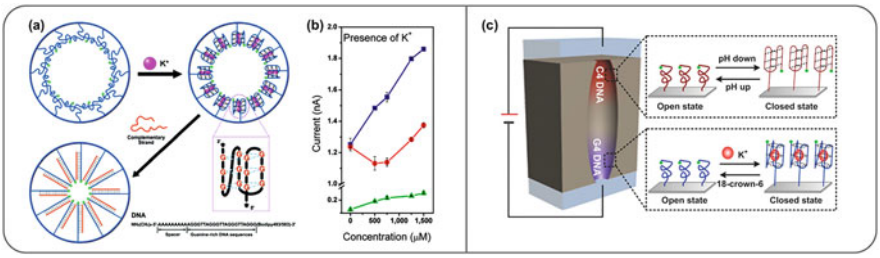

Fig. 3.4 (**a**) K^+-responsive G4 DNA. In the presence of K^+, the packed rigid quadruplex structures partially decrease the effective pore size. After adding complementary DNA strands, G4 DNA forms a closely packed arrangement of double-stranded DNA. (**b**) Current vs. K^+ concentration curve before G4 DNA modification (blue); after G4 DNA modification (red); after the addition of the complementary DNA strands (green). Reproduced with permission (Wang et al. 2009). Copyright © 2009 American Chemical Society. (**c**) Schematic illustration of the bio-inspired potassium and pH cooperative double-gated nanochannel. Reproduced with permission (Liu et al. 2015). Copyright © 2014 WILEY-VCH Verlag GmbH & Co

2016). Because of its simplicity and robustness, hybridization is the basis of many DNA analysis and diagnostic methods, in many cases in combination with amplification techniques to increase its sensitivity (Khodakov et al. 2016). In 2005, Vlassiouk et al. reported a pioneering work that used hybridization as a strategy to detect target DNA oligomers with AAO membranes (Vlassiouk et al. 2005). From that moment on, many attempts have been made to build nucleic acid sensors based on a hybridization mechanism in different types of nanochannels.

DNA Oligonucleotides

An illustrative example of a track-etched membrane-based DNA biosensor was developed by Sun et al. in 2016 (Sun et al. 2016). The authors reported the construction of nanofluidic channels for label-free, ultrasensitive, and highly sequence-specific detection of DNA (Fig. 3.5). The DNA probe oligonucleotides (bioreceptors) were immobilized by self-assembly on PEI-modified conical track-etched PET multi-SSNs. After that, BSA was adsorbed in order to avoid further nonspecific interactions with other negatively charged analytes (Fig. 3.5(a) and (b)). The as-prepared SSNs presented a near ohmic behavior due to their almost zero surface charge density. DNA detection was evaluated by monitoring the rectification efficiency of the diode-like behavior upon hybridization. A strong increment in the rectification efficiency was found when increasing the target complementary DNA sequence concentration, which could be explained by the increment in the net surface charge of the SSN that yields a binding-type dependency of the rectification factor on the target concentration (Fig. 3.5(c)). This device was able to discriminate complementary DNA (c-DNA) from noncomplementary DNA (nc-DNA) and one-base mismatched DNA (1bm-DNA) samples with high specificity (Fig. 3.5 (d)), even in real serum samples.

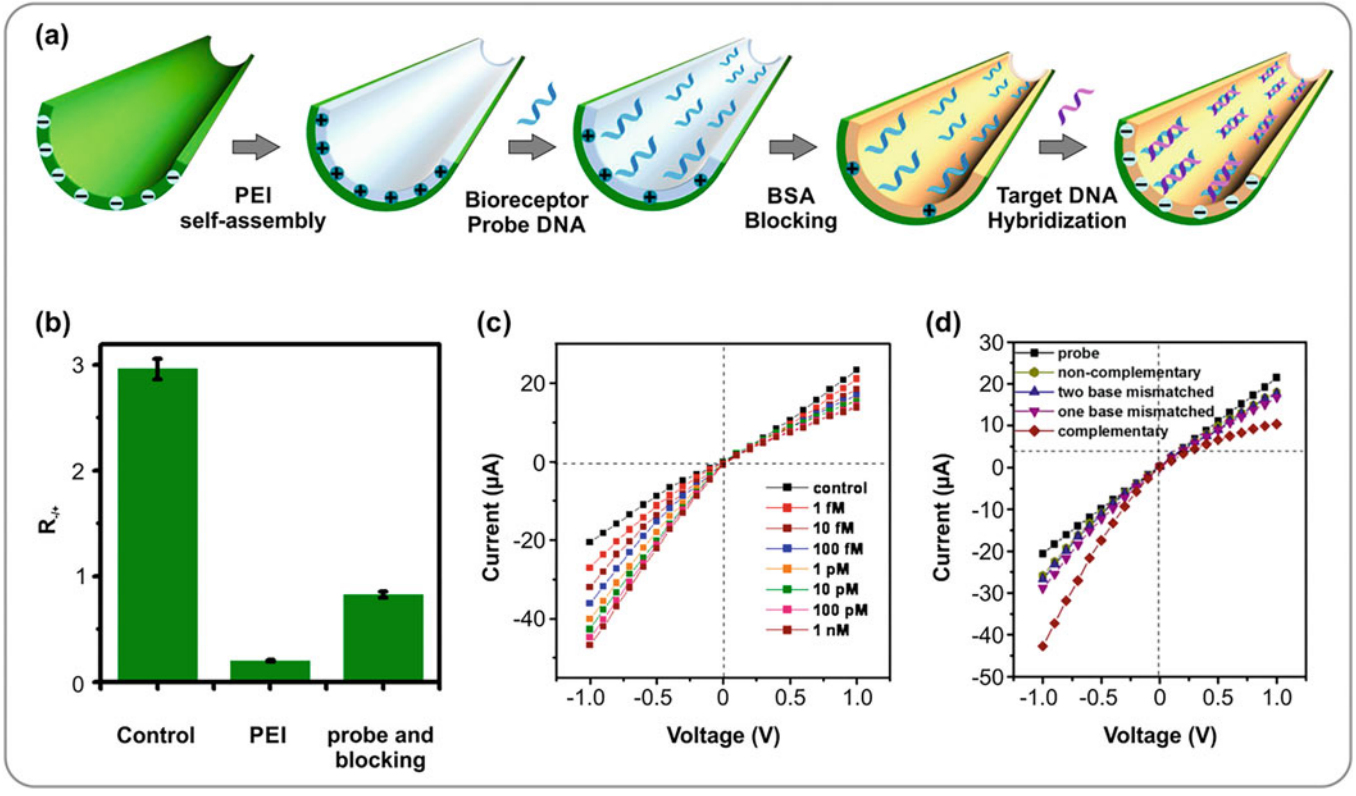

Fig. 3.5 (**a**) Schematic illustration of the probe DNA-functionalized PET nanochannel biosensor construction for DNA detection. (**b**) Rectifying characteristics of the layer-by-layer assembly process on the conical nanochannel ($R_{-/+}$ is defined as the absolute value of the current at −1 V divided by the current at +1 V). (**c**) *I–V* curves for different c-DNA concentrations. (**d**) *I–V* curves of the nanochannels after treated with 1 nM nc-DNA, 2bm-DNA, 1bm-DNA, and c-DNA. Adapted with permission (Sun et al. 2016). Copyright © 2016, Elsevier

DNA Superstructures

Further than double-strand hybridization, DNA superstructures have been utilized to create more efficient gating mechanisms in nanochannels (Pérez-Mitta et al. 2019). An instructive example was reported by Jiang and co-workers, who achieved the sub-nanomolar sequence-specific DNA detection (Liu et al. 2013). Track-etched PET nanochannel membranes were modified with predesigned capture DNA probes, and, in the presence of the target DNA, alternating units of signal probes (denoted as S1 and S2) were successively hybridized, bringing long concatamers (Fig. 3.6(a)). These DNA superstructures efficiently block ionic transport across the membrane, leading to a decrease in the transmembrane ionic current (Fig. 3.6(b)). Moreover, the DNA probe was modified with an aptamer sequence so that the presence of small target molecules caused the disassembly of the preformed supersandwich structures (Fig. 3.6(a) and (b)). With this strategy, the detection of oligonucleotides and ATP was successfully achieved.

Aptamers

Aptamers are nucleic acid molecules that can be rationally designed and tailored for a specific target (Dunn et al. 2017). Its use is promising because these molecules can be engineered into sensors, actuators, and other devices with relatively simple methods, which is central to emerging technologies. In this context, Li et al. reported the creation of a bioinspired adenosine artificial regulatory receptor (ARR) based on the self-assembly of designed adenosine (AD) aptamers onto gold-sputtered PET multi-nanochannels (Li et al. 2016). The selected recognition element of the ARR was a sequence-specific aptamer (SSA) that binds adenosine with a high affinity. In terms of the iontronic output, SSA self-assembly produced very low ionic currents (OFF state) due to the randomly stretched single-stranded structure of SSA that partially blocked the tip of the nanochannel. The exposition to AD and its binding to the SSA induced a conformational change in the aptamer that opened the pore (ON state). More specifically, AD and SSA formed beanpod structures that, in terms of ion transport, showed an increase in the ionic current (ON state).

DNA Hydrogels by the Hybridization Chain Reaction (HCR)

The concept of hybridization chain reaction (HCR) was introduced by Dirks and Pierce in 2004 (Dirks and Pierce 2004). They reported the mechanism by which stable DNA monomers (hairpins H1 and H2) assembled only upon exposition to a target single-stranded DNA fragment. Hairpins play the role of monomer DNA building blocks that hybridize only when an initiator strand (target DNA) triggers a polymerization reaction into a nicked double helix. The amplification of the initiator recognition event continues until H1 or H2 is completely consumed. Using HCR strategy, smart DNA hydrogel-functionalized potassium-responsive nanochannels were developed (Wu et al. 2018). For this aim, a PET membrane with a single conical channel was coated on the tip side with an Au film and then functionalized with a thiolated nucleic acid (initiator) (Fig. 3.7(a)). Then, DNA hydrogels were self-assembled via HCR using a series of hairpins (Fig. 3.7(a) and (b)). The DNA hydrogel-modified channel displayed a high rectification factor due

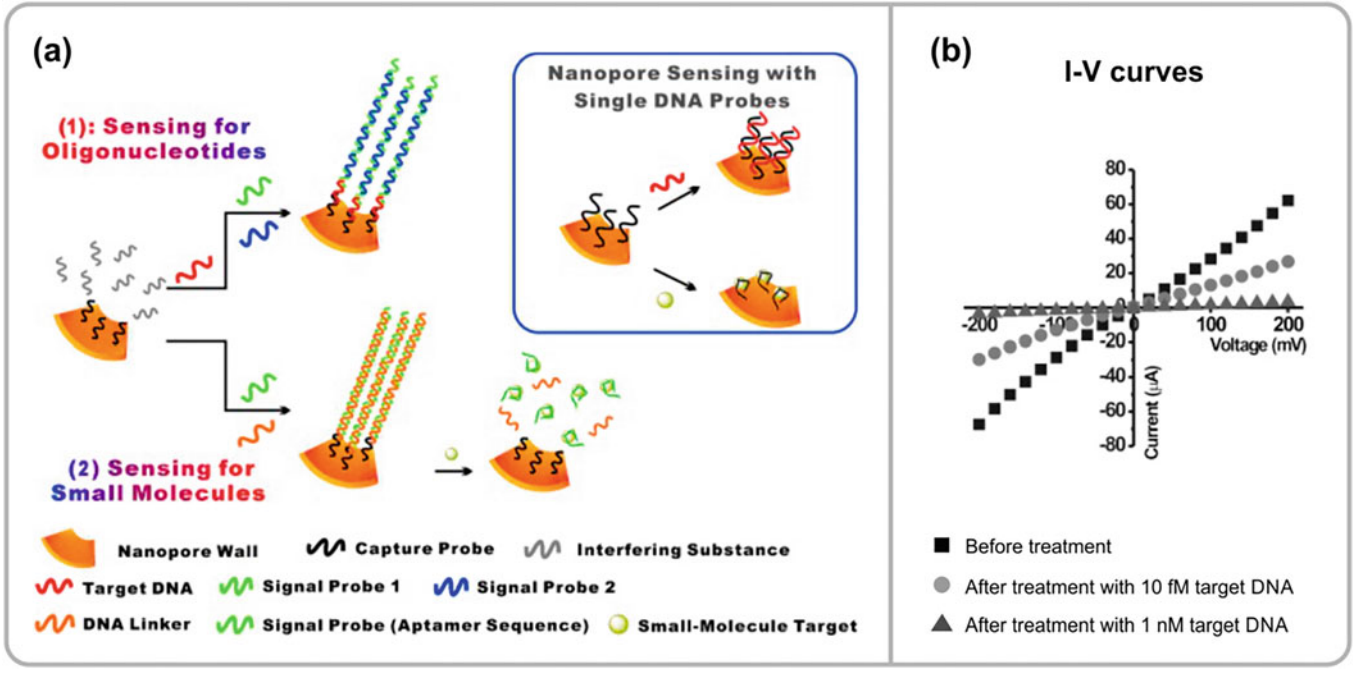

Fig. 3.6 (**a**) Improved biosensing strategy for oligonucleotides and small molecules. (**b**) Detection of DNA as monitored by nanopore conductance: *I–V* curves before (black small square) and after treatment with 10 fM (black small circle) or 1 nM (black up-pointing small triangle) of target DNA. Adapted with permission (Liu et al. 2013). Copyright © 2013, John Wiley & Sons, Inc.

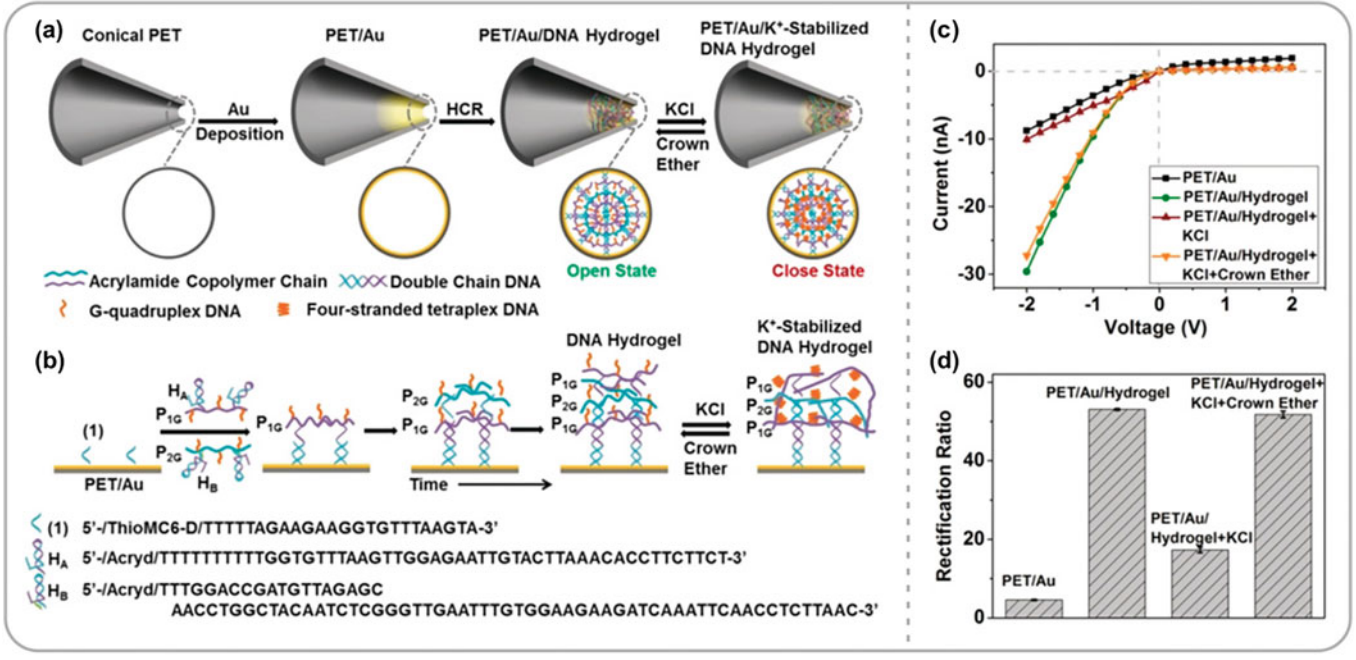

Fig. 3.7 (**a**) Scheme depicting the preparation process of the DNA hydrogels-based ion channel. (**b**) Detailed formation and transition of DNA hydrogel networks. (**c**) I–V curves and (**d**) rectification ratios of the conical nanochannel under different conditions (0.1 M LiCl, 10 mM Tris-HCl buffer, pH 7). Reproduced with permission (Wu et al. 2018). Copyright © 2018 John Wiley & Sons, Inc.

to the hydrophilicity and negative charge provided by the hydrogel (OPEN state) (Fig. 3.7(c) and (d)). When exposing the system to K^+ ions (0.05–1 M), a decrease in the rectification factor was observed (CLOSE state). Besides, by treating with 18-crown-6-ether solutions, K^+ ions could be released, leading to a softening of the DNA hydrogel structure.

Integration of DNAzymes

DNAzymes, also identified as deoxyribozymes or DNA enzymes, denote single-stranded DNA molecules with catalytic capabilities. In the last years, many efforts have been assumed to assess a variety of DNAzymes for innovation-driven applications such as gene regulation (Achenbach et al. 2004). Also, it is known that ions play an important role in maintaining normal physiological conditions and regulating several biological processes in living organisms (Hille 2001). Here, we present some relevant advances in the development of ion sensors based on DNAzyme-biofunctionalized track-etched nanochannels (Guo et al. 2019; Chen et al. 2016; Tu et al. 2021).

Chen et al. reported the construction of a nanofluidic device with Cu^{2+}-modulated ion transport properties by integrating a specific Cu^{2+}-induced self-cleaving DNAzyme into a polymer multi-nanochannel membrane (Chen et al. 2016). The creation included the nanochannel surface grafting with a short DNA sequence (DNA(2)) partially complementary to a DNAzyme (DNA(1)). Thus, DNA(2) partially hybridizes DNA(1) to form a double-stranded DNA (dsDNA). The DNAzyme-modified channel showed a diode-like behavior characterized by a negative surface as a consequence of the high negative charge provided by dsDNA. When exposing the membrane to Cu^{2+}, a decrease in the rectification factor was observed, in line with a Cu^{2+}-promoted break of DNA(1) into three fragments and its subsequent dissolution, which produced a decrease of the negative charge density.

Another example of a Zn^{2+} detection system based on cylindrical PET multichannel modified with both DNA supersandwich structure and Zn^{2+}-requiring DNAzyme was developed by Liu et al. (Liu et al. 2016) A capture probe, a sessile probe, a DNAzyme, and auxiliary DNA probes were immobilized at the nanochannel surface. The as-prepared DNA-modified channel showed an ohmic behavior with low conductance ascribed to the partial occlusion of the channel. On the contrary, in the presence of Zn^{2+}, a cleavage of the sessile probe and the stripping of the DNA supersandwich from the DNAzyme structure occur, leading to an increase in the conductance.

3.4.2.2 Proteins and Enzymes

In 2016, Ali et al. immobilized bovine serum albumin (BSA) on the surface of a single asymmetric nanochannel to develop a tryptophan (L-Trp) sensor (Ali et al. 2016). BSA molecules serve as chiral receptors since they enantioselectively recognize Trp over D-Trp through specific BSA–L-tryptophan interactions. In order to provide the channel with functionalization sites for BSA, dopamine (DA) was self-polymerized, allowing the deposit of a polydopamine (PDA) thin film onto the channel surface (Fig. 3.8(a)). Then, BSA amine groups reacted with PDA through

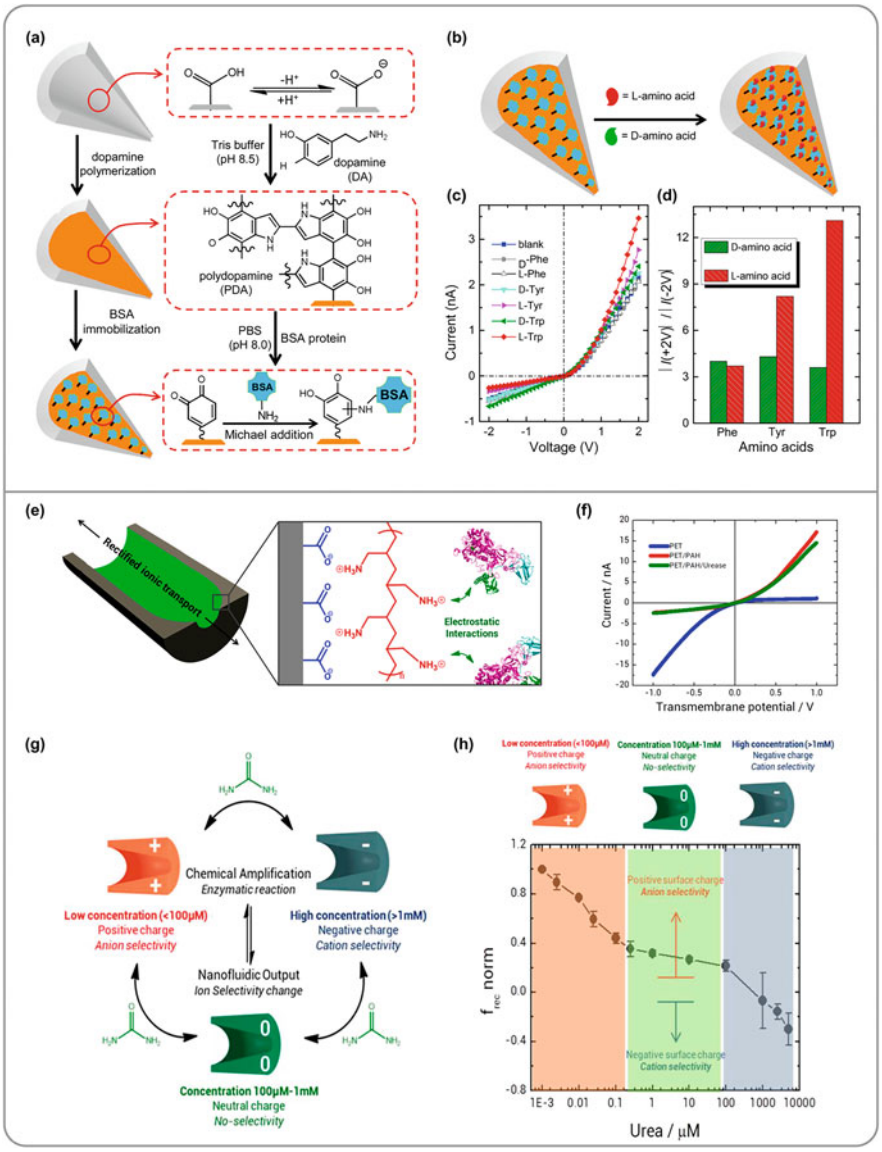

Fig. 3.8 (**a**) Functionalization of the PET nanochannel for L-tryptophan biosensing. (**b**) Specific binding of amino acid enantiomer onto BSA-modified nanochannel surface. (**c**) I–V curves of the BSA-modified SSN before (blank) and after exposure to 1.5 mM solution of D-/L-Phe, D-/L-Tyr, and D-/L-Trp (100 mM KCl, pH 5.8). (**d**) Changes in the rectification factor (f_{rec}) at 2 V. Reproduced with permission (Ali et al. 2016). Copyright © 2016 Elsevier B.V. (**e**) Urease-modified single PET nanochannel. (**f**) *I–V* curves before and after each modification step at pH 6. (**g**) Scheme of the biochemical degradation of urea and the nanofluidic output of the nanochannel through the amplification of the enzymatic reaction. (**h**) Calibration curve in terms of normalized rectification factor *vs* urea concentration. Reproduced with permission (Pérez-Mitta et al. 2018b). Copyright © 2018, American Chemical Society

the Michael addition reaction. In terms of ion transport, the specific BSA–L-tryptophan interactions led to changes in the surface charge density that modulated the ionic transport through the nanochannel (Fig. 3.8(b), (c) and (d)).

Even more, the integration of enzymatic processes into SSNs confers them outstanding specificity on target analytes as a result of biological recognition. An illustrative example was provided in a pioneering work by Pérez-Mitta et al. (Pérez-Mitta et al. 2018b) The authors provided evidence on the mechanism for sensing urea by using a urease-modified single PET nanochannel. First, the electrostatic assembly of poly(allylamine) (PAH) on the etched bullet-shaped PET nanochannels was performed, and afterward urease was electrostatically adsorbed on the PAH-modified channel (Fig. 3.8(e) and (f)). Urease catalyzes the hydrolysis of urea into ammonia and carbon dioxide, causing a local pH increase and, thus, a decrease in the protonation degree of the PAH, reducing the positive surface charge density in the channel. This biosensing device was able to reach a limit of detection of 1 nM of urea (Fig. 3.8(g) and (h)).

3.4.2.3 Other Biomolecules

Amino Acids

Recently, Lou et al. reported a new L-tyrosine functionalized SSN-based strategy to detect H_2O_2 released from living cells without insertion procedures (Lou et al. 2020). First, cervical cancer living cells (HeLa) were incubated on a PET membrane with cylindrical nanochannels. After that, the PET surface carboxyl groups were functionalized by coupling chemistry with L-tyrosine-generating phenolic hydroxyl groups on the nanochannel surface. Afterward, the addition of an aggregation-induced emission luminogen (TT) in the presence of horseradish peroxidase (HRP) and H_2O_2 released from living cells derived in the formation of di-tyrosine linkages and TT oligomerization. This fact provoked a partial blocking of the ion transport pathway, which was detected by ion transport measurements. A high sensitivity response to H_2O_2 was obtained as, for 1 nM H_2O_2, a resistance of more than 100 kΩ was measured (+0.2 V).

3.5 Conclusions and Outlook

Solid-state nanochannels have become a versatile platform for the design and development of biosensing devices based on nanofluidics. Due to the influence of the ionic surface charge and the effective channel size on the ionic current through the nanochannels, the functional coupling of biorecognition entities on the nanochannel surface and their specific interaction with target molecules affect the recorded ionic current due to the caused changes in ionic surface charge and/or effective channel size, simultaneously enabling specific responsiveness and sensing. Some biological molecules, such as nucleic acids and enzymes, are able to exhibit interactions with some specific targets with high efficiency, but they need to be properly integrated into the confined environment of the inner surface of the

nanochannels. In this chapter, the basis of the application of SSNs for biosensing purposes, including the nanochannel fabrication and the transduction of iontronic signals, was presented together with some illustrative examples of how the proper integration of biorecognition elements within the inner surface of the channels allows sensing and quantifying a variety of analytes in solution from the changes in the iontronic response.

Due to the nanometer size of the active sensor components, i.e., the nanochannels, the miniaturization of the SSN-based technology should become straightforward, naturally heading toward the use of SSNs for ultrasensitive detection and quantification of biomarkers and drugs for clinical, pharmaceutical, and forensics applications in miniaturized devices.

References

Achenbach J, Chiuman W, Cruz R, Li Y (2004) DNAzymes: from creation in vitro to application in vivo. Curr Pharm Biotechnol 5:321

Ali M, Yameen B, Neumann R, Ensinger W, Knoll W, Azzaroni O (2008) Biosensing and Supramolecular Bioconjugation in Single Conical Polymer Nanochannels. Facile Incorporation of Biorecognition Elements into Nanoconfined Geometries. J Am Chem Soc 130:16351

Ali M, Neumann R, Ensinger W (2010a) Sequence-specific recognition of DNA oligomer using peptide nucleic acid (PNA)-modified synthetic ion channels: PNA/DNA hybridization in nanoconfined environment. ACS Nano 4:7267

Ali M, Yameen B, Cervera J, Ramírez P, Neumann R, Ensinger W, Knoll W, Azzaroni O (2010b) Layer-by-layer assembly of polyelectrolytes into ionic current rectifying solid-state nanopores: insights from theory and experiment. J Am Chem Soc 132:8338

Ali M, Yameen B, Cervera J, Ramírez P, Neumann R, Ensinger W, Knoll W, Azzaroni O, Ramı P, Neumann R, Ensinger W, Knoll W, Azzaroni O (2010c) Layer-by-layer assembly of polyelectrolytes into ionic current rectifying solid-state nanopores: insights from theory and experiment. J Am Chem Soc 132:8338

Ali M, Nasir S, Ensinger W (2016) Stereoselective detection of amino acids with protein-modified single asymmetric nanopores. Electrochim Acta 215:231

Apel PY, Fink D (2004) In: Fink D (ed) Transport processes in ion-irradiated polymers. Springer-Verlag, Berlin/Heidelberg, pp 147–202

Apel PY, Blonskaya IV, Dmitriev SN, Orelovitch OL, Presz A, Sartowska BA (2007) Fabrication of nanopores in polymer foils with surfactant-controlled longitudinal profiles. Nanotechnology 18:305302

Apel PY, Blonskaya IV, Levkovich NV, Orelovich OL (2011) Asymmetric track membranes: relationship between nanopore geometry and ionic conductivity. Pet Chem 51:555

Bard AJ, Faulkner LR (2001) Electrochemical methods. Fundamentals and applications. Wiley

Brett CMA, Oliveira Brett AM (1993) Electrochemistry: principles, methods, and applications. Oxford University Press

Buchsbaum SF, Nguyen G, Howorka S, Siwy ZS (2014) DNA-modified polymer pores allow pH-and voltage-gated control of channel flux. J Am Chem Soc 136:9902

Cervera J, Schiedt B, Neumann R, Mafé S, Ramírez P (2006) Ionic conduction, rectification, and selectivity in single conical nanopores. J Chem Phys 124:104706

Chen Y, Zhou D, Meng Z, Zhai J (2016) An ion-gating multinanochannel system based on a copper-responsive self-cleaving DNAzyme. Chem Commun 52:10020

De Leo M, Pereira FC, Moretto LM, Scopece P, Polizzi S, Ugo P (2007) Towards a better understanding of gold electroless deposition in track-etched templates. Chem Mater 19:5955

Decher G, Hong JD, Schmitt J (1992) Buildup of ultrathin multilayer films by a self-assembly process: III. Consecutively alternating adsorption of anionic and cationic polyelectrolytes on charged surfaces. Thin Solid Films 210–211:831

Ding D, Gao P, Ma Q, Wang D, Xia F (2019) Biomolecule-functionalized solid-state ion nanochannels/nanopores: features and techniques. Small 15:1804878

Dirks RM, Pierce NA (2004) Triggered amplification by hybridization chain reaction. Proc Natl Acad Sci U S A 101(43):15275–15278

Dunn MR, Jimenez RM, Chaput JC (2017) Analysis of aptamer discovery and technology. Nat Rev Chem 1:0076

Gao J, Guo W, Feng D, Wang H, Zhao D, Jiang L (2014) High-performance ionic diode membrane for salinity gradient power generation. J Am Chem Soc 136:12265

George SM (2010) Atomic layer deposition: an overview. Chem Rev 110:111

Gilles FM, Boubeta FM, Azzaroni O, Szleifer I, Tagliazucchi M (2018) Modulation of polyelectrolyte adsorption on nanoparticles and nanochannels by surface curvature. J Phys Chem C 122: 6669

Gleich A, Pade C, Petschow U, Pissarskoi E (2010) Potentials and trends in biomimetics. Springer, Berlin, Heidelberg

Guo J, Yang L, Xu H, Zhao C, Dai Z, Gao Z, Song Y (2019) Biomineralization-driven ion gate in TiO_2 nanochannel arrays for cell H_2S sensing. Anal Chem 91:13746

Harrell CC, Kohli P, Siwy Z, Martin CR (2004) DNA-nanotube artificial ion channels. J Am Chem Soc 126:15646

Hille B (2001) Ion channels of excitable membranes. Sinauer Associates, Sunderland, MA

Khodakov D, Wang C, Zhang DY (2016) Diagnostics based on nucleic acid sequence variant profiling: PCR, hybridization, and NGS approaches. Adv Drug Deliv Rev 105:3–19

Kumari B, John D, Hoffmann P, Spende A, Toimil-Molares ME, Trautmann C, Hess C, Ruff P, Schulze M, Stark R, Buntkowsky G, Andrieu-Brunsen A, Gutmann T (2018) Surface enhanced DNP assisted solid-state NMR of functionalized SiO_2 coated polycarbonate membranes. Z Phys Chem 232:1173

Laucirica G, Marmisollé WA, Toimil-Molares ME, Trautmann C, Azzaroni O (2019a) Redox-driven reversible gating of solid-state nanochannels. ACS Appl Mater Interfaces 11:30001

Laucirica G, Pérez-Mitta G, Toimil-Molares MEME, Trautmann C, Marmisollé WAWA, Azzaroni O (2019b) Amine-phosphate specific interactions within nanochannels: binding behavior and nanoconfinement effects. J Phys Chem C 123:28997

Laucirica G, Cayón VM, Toum Terrones Y, Cortez ML, Toimil-Molares ME, Trautmann C, Marmisollé WA, Azzaroni O (2020a) Electrochemically addressable nanofluidic devices based on PET nanochannels modified with electropolymerized poly-o-aminophenol films. Nanoscale 12:6002

Laucirica G, Toimil-Molares ME, Trautmann C, Marmisollé WA, Azzaroni O (2020b) Polyaniline for improved blue energy harvesting: highly rectifying nanofluidic diodes operating in hypersaline conditions via one-step functionalization. ACS Appl Mater Interfaces 12:28148

Laucirica G, Albesa AG, Toimil-Molares ME, Trautmann C, Marmisollé WA, Azzaroni O (2020c) Shape matters: enhanced osmotic energy harvesting in bullet-shaped nanochannels. Nano Energy 71:104612

Laucirica G, Toum Terrones Y, Cayón V, Cortez ML, Toimil-Molares ME, Trautmann C, Marmisollé WA, Azzaroni O (2020d) High-sensitivity detection of dopamine by biomimetic nanofluidic diodes derivatized with poly(3-aminobenzylamine). Nanoscale 12:18390

Lee YH, Mutharasan R (2005) In: Wilson JS (ed) Sensor technology handbook. Elsevier, pp 161–180

Li P, Kong X-Y, Xie G, Xiao K, Zhang Z, Wen L, Jiang L (2016) Adenosine-activated nanochannels inspired by G-protein-coupled receptors. Small 12:1854

Liu N, Jiang Y, Zhou Y, Xia F, Guo W, Jiang L (2013) Inside cover: two-way nanopore sensing of sequence-specific oligonucleotides and small-molecule targets in complex matrices using

integrated DNA supersandwich structures (Angew. Chem. Int. Ed. 7/2013). Angew Chemie - Int Ed 52:1842
Liu M, Zhang H, Li K, Heng L, Wang S, Tian Y, Jiang L (2015) A bio-inspired potassium and pH responsive double-gated nanochannel. Adv Funct Mater 25:421
Liu N, Hou R, Gao P, Lou X, Xia F (2016) Sensitive Zn^{2+} sensor based on biofunctionalized nanopores via combination of DNAzyme and DNA supersandwich structures. Analyst 141: 3626
Lou X, Song Y, Liu R, Cheng Y, Dai J, Chen Q, Gao P, Zhao Z, Xia F (2020) Enzyme and AIEgens modulated solid-state nanochannels: in situ and noninvasive monitoring of H_2O_2 released from living cells. Small Methods 4:1900432
Love JC, Estroff LA, Kriebel JK, Nuzzo RG, Whitesides GM (2005) Self-assembled monolayers of thiolates on metals as a form of nanotechnology. Chem Rev 105:1103
Ma T, Gaigalas P, Lepoitevin M, Plikusiene I, Bechelany M, Janot J-M, Balanzat E, Balme S (2018) Impact of polyelectrolyte multilayers on the ionic current rectification of conical nanopores. Langmuir 34:3405
Martin CR, Nishizawa M, Jirage K, Kang M, Lee SB (2001) Controlling ion-transport selectivity in gold nanotubule membranes. Adv Mater 13:1351
Muench F, Kunz U, Neetzel C, Lauterbach S, Kleebe H-J, Ensinger W (2011) 4-(Dimethylamino) pyridine as a powerful auxiliary reagent in the electroless synthesis of gold nanotubes. Langmuir 27:430
Nasir S, Ali M, Ramirez P, Gómez V, Oschmann B, Muench F, Tahir MN, Zentel R, Mafe S, Ensinger W (2014) Fabrication of single cylindrical Au-coated nanopores with non-homogeneous fixed charge distribution exhibiting high current rectifications. ACS Appl Mater Interfaces 6:12486
Netz RR, Andelman D (2003) Neutral and charged polymers at interfaces. Phys Rep 380:1–95
Ohno I (1991) Electrochemistry of electroless plating. Mater Sci Eng A 146:33
Pérez-Mitta G, Tuninetti JS, Knoll W, Trautmann C, Toimil-Molares ME, Azzaroni O (2015a) Polydopamine meets solid-state nanopores: a bioinspired integrative surface chemistry approach to tailor the functional properties of nanofluidic diodes. J Am Chem Soc 137:6011
Pérez-Mitta G, Albesa AG, Knoll W, Trautmann C, Toimil-Molares ME, Azzaroni O (2015b) Host–guest supramolecular chemistry in solid-state nanopores: potassium-driven modulation of ionic transport in nanofluidic diodes. Nanoscale 7:15594
Pérez-Mitta G, Marmisollé WA, Trautmann C, Toimil-Molares ME, Azzaroni O (2015c) Nanofluidic diodes with dynamic rectification properties stemming from reversible electrochemical conversions in conducting polymers. J Am Chem Soc 137:15382
Pérez-Mitta G, Burr L, Tuninetti JS, Trautmann C, Toimil-Molares ME, Azzaroni O (2016) Noncovalent functionalization of solid-state nanopores via self-assembly of amphipols. Nanoscale 8:1470
Pérez-Mitta G, Albesa AG, Trautmann C, Toimil-Molares ME, Azzaroni O (2017a) Bioinspired integrated nanosystems based on solid-state nanopores: "*iontronic*" transduction of biological, chemical and physical stimuli. Chem Sci 8:890
Pérez-Mitta G, Albesa A, Gilles FM, Toimil-Molares ME, Trautmann C, Azzaroni O (2017b) Noncovalent approach toward the construction of nanofluidic diodes with ph-reversible rectifying properties: insights from theory and experiment. J Phys Chem C 121:9070
Pérez-Mitta G, Marmisollé WA, Trautmann C, Toimil-Molares ME, Azzaroni O (2017c) An all-plastic field-effect nanofluidic diode gated by a conducting polymer layer. Adv Mater 29: 1700972
Pérez-Mitta G, Marmisolle WA, Burr L, Toimil-Molares ME, Trautmann C, Azzaroni O (2018a) Proton-gated rectification regimes in nanofluidic diodes switched by chemical effectors. Small 14:1703144
Pérez-Mitta G, Peinetti AS, Cortez ML, Toimil-Molares ME, Trautmann C, Azzaroni O (2018b) Highly sensitive biosensing with solid-state nanopores displaying enzymatically reconfigurable rectification properties. Nano Lett 18:3303

Pérez-Mitta G, Toimil-Molares ME, Trautmann C, Marmisollé WA, Azzaroni O (2019) Molecular design of solid-state nanopores: fundamental concepts and applications. Adv Mater 31:1901483

Ruff P, Carrillo-Solano M, Ulrich N, Hadley A, Kluth P, Toimil-Molares ME, Trautmann C, Hess C (2018) Nanoscale structuring in confined geometries using atomic layer deposition: conformal coating and nanocavity formation. Z Phys Chem 232:1147

Sander MS, Côté MJ, Gu W, Kile BM, Tripp CP (2004) Template-assisted fabrication of dense, aligned arrays of titania nanotubes with well-controlled dimensions on substrates. Adv Mater 16:2052

Schoch RB, Renaud P (2005) Ion transport through nanoslits dominated by the effective surface charge. Appl Phys Lett 86:253111

Siwy ZS (2006) Ion-current rectification in nanopores and nanotubes with broken symmetry. Adv Funct Mater 16:735

Siwy Z, Apel P, Dobrev D, Neumann R, Spohr R, Trautmann C, Voss K (2003) Nuclear instruments and methods in physics research section B: beam interactions with materials and atoms. Elsevier BV 208:143

Sobel N, Hess C, Lukas M, Spende A, Stühn B, Toimil-Molares ME, Trautmann C (2015) Conformal SiO_2 coating of sub-100 nm diameter channels of polycarbonate etched ion-track channels by atomic layer deposition. Beilstein J. Nanotechnol. 6:472

Spende A, Sobel N, Lukas M, Zierold R, Riedl JC, Gura L, Schubert I, Moreno JMM, Nielsch K, Stühn B, Hess C, Trautmann C, Toimil-Molares ME (2015) TiO_2, SiO_2, and Al_2O_3 coated nanopores and nanotubes produced by ALD in etched ion-track membranes for transport measurements. Nanotechnol 26:335301

Spohr R (1990) Ion tracks and microtechnology: principles and applications. Vieweg+Teubner Verlag

Sun Z, Liao T, Zhang Y, Shu J, Zhang H, Zhang GJ (2016) Biomimetic nanochannels based biosensor for ultrasensitive and label-free detection of nucleic acids. Biosens Bioelectron 86:194

Tagliazucchi M, Szleifer I (2016) Chemically modified nanopores and nanochannels. Elsevier Inc.

Tan F, Leung PHM, Liu Z, Zhang Y, Xiao L, Ye W, Zhang X, Yi L, Yang M (2011) A PDMS microfluidic impedance immunosensor for E. coli O157: H7 and Staphylococcus aureus detection via antibody-immobilized nanoporous membrane. Sensors Actuators B Chem 159:328

Tian Y, Hou X, Wen L, Guo W, Song Y, Sun H, Wang Y, Jiang L, Zhu D (2010) A biomimetic zinc activated ion channel. Chem Commun 46:1682

Tian Y, Hou X, Jiang L (2011) Biomimetic ionic rectifier systems: asymmetric modification of single nanochannels by ion sputtering technology. J Electroanal Chem 656:231

Toimil-Molares ME (2012) Characterization and properties of micro- and nanowires of controlled size, composition, and geometry fabricated by electrodeposition and ion-track technology. Beilstein J Nanotechnol 3:860

Trautmann C (2009) In: Hellborg R, Whitlow HJ, Zhang Y (eds) Ion beams in nanoscience and technology. Springer-Verlag, Berlin/Heidelberg, pp 369–387

Trautmann C, Brüchle W, Spohr R, Vetter J, Angert N (1996) Pore geometry of etched ion tracks in polyimide. Nucl Instruments Methods Phys Res Sect B Beam Interact Mater Atoms 111:70

Tu J, Zhou Z, Liu Y, Li T, Lu S, Xiao L, Xiao P, Zhang G, Sun Z (2021) Nanochannel-based sensor for the detection of lead ions in traditional Chinese medicine. RSC Adv 11:3751

Vlassiouk I, Takmakov P, Smirnov S (2005) Sensing DNA hybridization via ionic conductance through a nanoporous electrode. Langmuir 21:4776

Vlassiouk I, Kozel TR, Siwy ZS (2009) Biosensing with nanofluidic diodes. J Am Chem Soc 131: 8211

Wang YG, Hou X, Guo W, Xia F, Nie FQ, Dong H, Tian Y, Wen LP, Wang L, Cao LX, Yang Y, Xue JM, Song YL, Liu DS, Jiang L (2009) A biomimetic potassium responsive nanochannel: G-quadruplex DNA conformational switching in a synthetic nanopore. J Am Chem Soc 131: 7800

Wu Y, Wang D, Willner I, Tian Y, Jiang L (2018) Smart DNA hydrogel integrated nanochannels with high ion flux and adjustable selective ionic transport. Angew Chemie Int Ed 57:7790

Yameen B, Ali M, Neumann R, Ensinger W, Knoll W, Azzaroni O (2009) Ionic transport through single solid-state nanopores controlled with thermally nanoactuated macromolecular gates. Small 5:1287

Yameen B, Ali M, Álvarez M, Neumann R, Ensinger W, Knoll W, Azzaroni O (2010) A facile route for the preparation of azide-terminated polymers. "Clicking" polyelectrolyte brushes on planar surfaces and nanochannels. Polym Chem 1:183

Zhang Z, Wen L, Jiang L (2018) Bioinspired smart asymmetric nanochannel membranes. Chem Soc Rev 47:322

Zhao S, Zheng Y-B, Cai S-L, Weng Y-H, Cao S-H, Yang J-L, Li Y-Q (2013) Sugar-stimulated robust nanodevice: 4-carboxyphenylboronic acid modified single glass conical nanopores. Electrochem Commun 36:71