Biosensors and Bioelectronics 60 (2014) 92–100

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

An ion-exchange nanomembrane sensor for detection of nucleic acids using a surface charge inversion phenomenon

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ARTICLE INFO

Article history:
Received 21 January 2014
Received in revised form
1 April 2014
Accepted 2 April 2014
Available online 13 April 2014

Keywords:
Biosensing
DNA/RNA
Electrokinetics
Charge inversion
Nanoporous membrane

ABSTRACT

We present a novel low-cost biosensor for rapid, sensitive and selective detection of nucleic acids based on an ionic diode feature of an anion exchange nanoporous membrane under DC bias. The ionic diode feature is associated with external surface charge inversion on the positively charged anion exchange nanomembrane upon hybridization of negatively charged nucleic acid molecules to single-stranded oligoprobes functionalized on the membrane surface resulting in the formation of a cation selective monolayer. The resulting bipolar membrane causes a transition from electroconvection-controlled to water-splitting controlled ion conductance, with a large ion current signature that can be used to accurately quantify the hybridized nucleic acids. The platform is capable of distinguishing two base-pair mismatches in a 22-base pairing segment of microRNAs associated with oral cancer, as well as serotype-specific detection of dengue virus. We also show the sensor's capability to selectively capture target nucleic acids from a heterogeneous mixture. The limit of detection is 1 pM for short 27 base target molecules in a 15-min assay. Similar hybridization results are shown for short DNA molecules as well as RNAs from Brucella and *Escherichia coli*. The versatility and simplicity of this low-cost biosensor should enable point-of-care diagnostics in food, medical and environmental safety markets.

1. Introduction

DNA and RNA-based pathogen diagnostics remain as one of the most active fields of nucleic acid research (Gingeras et al., 2005; Wei et al., 2010). PCR amplification remains a gold standard in nucleic acid-based diagnosis, but it requires expensive labels and trained personnel – and is time consuming because of multiple steps involved. In recent years, there has been interest in developing probe-based non-optical sensors that can obviate the use of PCR and fluorophore labeling to improve the detection time and lower the cost of diagnostic tests. A large number of papers have been published on amplification-free nucleic acid biosensors employing different transduction sensing mechanisms (Drummond et al., 2003; O'Connor and Glynn, 2010; Palchetti and Mascini, 2008; Peng and Miller, 2011) of which label-free technologies are of special interest (Kataoka-Hamai and Miyahara, 2011; Ricci and Plaxco, 2008).

Nucleotides in DNA/RNA molecules are linked together through a sugar-phosphate backbone. The presence of the phosphate groups in this backbone renders both DNA and RNA molecules negatively charged making them suitable for manipulation (e.g. gel electrophoresis) as well as detection under electrical field. This intrinsic negative charge of DNA/RNA molecules can have a profound effect on both electronic and ionic conductivity of a system and has been explored to develop label-free nucleic acid sensors by measuring the electrical signal of the system. One such example is the field-effect-transistor (FET) that uses the electronic conductivity of the system for the detection of DNA molecules. In this system, the current passing between a source and a drain is controlled by the potential connected to a gate. This potential is sensitive to the negative charge of nucleic acid molecules present within the Debye length of the gate (Fritz et al., 2002). This changes the capacitance of the gate–electrolyte interface resulting in a change in the total current passing through the transistor (Kim et al., 2004; Pandana et al., 2008). Further, Li et al. (2004)

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http://dx.doi.org/10.1016/j.bios.2014.04.008
0956-5663/

demonstrated the detection of DNA by monitoring the change in conductivity of silicon nanowires.

Researchers have also demonstrated nucleic acid detection by directly monitoring the influence of DNA/RNA molecules on the ionic conductivity of nanochannels. These nanochannels are often single nanopores or an array of nanopores, where the charge and conductance of the nucleic acids within the nanochannels affects the intra-channel ion conductance. The immobilized DNA can render the nanochannel, or part of it, ion selective (Hou et al., 2013). Jagerszki et al. (2007) measured flux of easily traceable anionic dye in a nanochannel functionalized with neutral peptide nucleic acid (PNA) probe and its hybridization to target DNA. While the PNA probe did not hinder the flux of anionic dye, the hybridized target DNA molecules efficiently decreased the flux through the channel. Further, Ali et al. (2010) observed a significant change in current voltage characteristics (CVC) upon hybridization of target DNA to uncharged peptide nucleic acid probes functionalized to a conical nanopore. Wang and Smirnov (2009) employed an ion selective alumina nanochannel for the detection of DNA molecules and used the change in conductivity of nanochannel upon DNA hybridization as a detection signal. Other groups have demonstrated sensors involving biological nanopores where DNA hybridization causes opening or closing of the nanopore which can be tracked by measuring the ionic current going through the nanopore (Krishnamurthy et al., 2010a, 2010b; Steller et al., 2012). This switch mechanism can essentially be used to detect any biomolecules including proteins and nucleic acids (Lucas and Harding, 2000). A similar but simpler approach was presented by WeissWichert et al. (1997) where the binding of the target molecules blocked the entrance to the gramidicin pore resulting in diminished flux of ions.

Both FET and nanochannel sensors remain expensive to fabricate – and hence have not been commercialized for field-use diagnostics. Their small current (nA) and voltage (mV) signals also render both platforms sensitive to noise and contamination. While translocation time for single molecule through a nanochannel is short (ms), the total time to interrogate every molecule in the sample is still prohibitively long. Herein we report a novel, low cost and label-free biosensing platform for the detection of negatively charged nucleic acids using a positively-charged, heterogeneous anion exchange nanoporous membrane (Fig. 1A). The sensor is based on a charge inversion phenomenon (Slouka et al., 2013) that occurs on the surface of a positively charged nanomembrane (not within the nanopores of the membrane) when negatively charged nucleic acid molecules bind to its surface. It operates at a much higher voltage than the nanochannel sensors because ion conductance is controlled by the surface charge of the membrane surface and two unique non-equilibrium ion transport phenomena described later. Anion exchange membranes are known to exhibit interesting non-linear current–voltage characteristics (Fig. 1B – black curve) that arise due to the differences in fluxes of ions in the solution and the membrane. Small counter-ions (anions) are the main carriers of ionic current in the positively charged membrane (Fig. 1C), while large anions and cations do not contribute to this ionic current due to size exclusion and electrostatic repulsion effects respectively. At low voltages, the current increases linearly with voltage showing an Ohmic behavior which is also known as the underlimiting region on the CVC (Fig. 1B black curve). Nanochannel sensors operate in this low-voltage Ohmic region. With increasing voltage, the concentration of the electrolyte on one side of the nanomembrane decreases (depletion side) while the concentration of the electrolyte on the other side

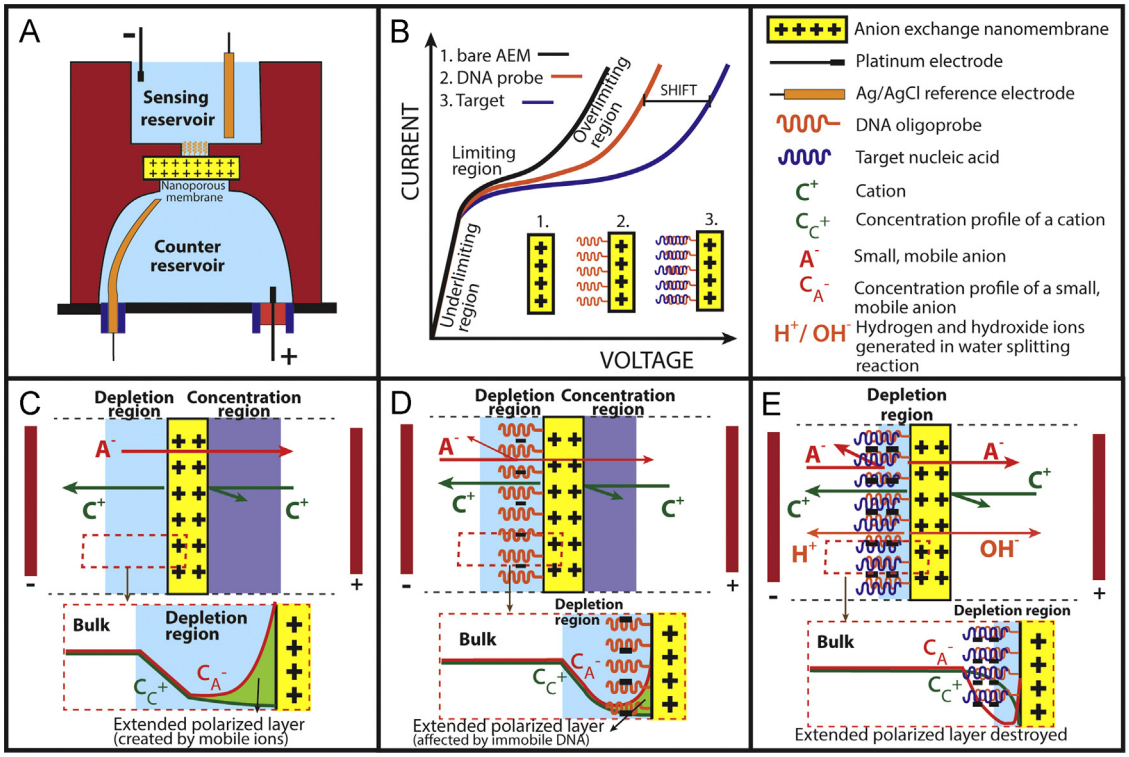

Fig. 1. Schematics and working principle of the nanomembrane sensor. (A) Diagrammatic representation of a nanomembrane electrokinetic sensor consisting of the top sensing reservoir and bottom counter reservoir bridged together by a positively charged nanomembrane. (B) Current–voltage characteristics (CVC) showing changes in Ohmic, limiting and overlimiting regions for bare anion-exchange membrane (black), membrane functionalized with oligoprobe (red) and hybridization of DNA/RNA with oligoprobe (blue). We utilize these changes in CVC for detection of nucleic acids. (C) Mechanism for the Ohmic relationship at low voltages known as the underlimiting regions. (D) Changes in CVC as a result of DNA adsorption on the membrane surface leading to changes in the limiting regions. (E) Mechanism for the overlimiting region at high DNA concentrations causing electroconvection and water splitting phenomenon. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)