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Nanoparticle displacement assay with electrochemical nanopore-based sensors

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ABSTRACT

The proof of concept of a nanoparticle displacement assay that enables the use of large diameter nanopores for the detection of targets of smaller molecular dimensions is presented. We hypothesized that an inherent signal amplification should arise from the selective displacement of nanoparticles preloaded in a nanopore by a much smaller molecular target. The method is demonstrated using peptide nucleic acid (PNA)-functionalized gold nanopore arrays in which short DNA-modified gold nanoparticles are anchored by weak interaction. Complementary microRNAs are detected via the resistance change caused by competitive displacement of nanoparticles from the PNA-functionalized nanopores.

1. Introduction

Electrochemical nanopore sensors are based on detecting changes caused by target species in the physical-chemical properties, most often electrical conductivity, of the minute space delimited by the nanopore interior [1–5]. This sensing concept with single species detection capability has been studied for the determination of a wide variety of targets ranging from small ions [6] up to nanoparticles [6–12]. While the size resolution of nanopore-based resistive pulse sensors can reach subnanometer levels [13], their analytical use, i.e. selective detection of target species in complex samples, still requires the use of selective receptors, either added to the sample solution [10,14] or confined to the nanopore interior [15,16]. In this latter approach either stochastic sensing [17,18] based on reversible binding of the target [19,20] or the more conventional approach in which the target is bound "irreversibly" into the nanopore sensing zone is used [21–25]. While there are exceptions [20], nanopore sensing requires nanopores with sizes that are comparable to, but larger than, that of the target. For solid state nanopores this requirement means that the smaller the target species the more demanding becomes the nanopore fabrication process in terms of reliability and cost. Therefore, we became interested in exploring sensing schemes that are able to address this problem, i.e., which have larger diameter pores adapted for the detection of much smaller macromolecules. Here we report the proof of concept of a novel nanoparticle-based displacement assay using solid-state nanopore arrays to amplify the signal of oligonucleotides, taking as a model the detection of a 22-mer microRNA, miR-208a [26], a potential biomarker of acute myocardial infarction. For this purpose we functionalized gold nanopore arrays made by focused ion beam (FIB) milling with a thiolated 18-mer peptide nucleic acid (PNA) probe that is uncharged and binds with high affinity to the complementary miR-208a [27]. Before analysis the PNA-functionalized nanopores were filled with 13 nm diameter gold nanoparticles (AuNPs) modified with 10-mer thiolated DNA (DNA-AuNP) - complementary to PNA - to block the ion current through the nanopores (Scheme 1). The shorter DNA strands were designed to have a weak interaction with the PNA layer so that DNA-AuNPs can be released by the competitive action of the miR-208a strands. We anticipated that the release of the nanoparticles, which are much larger than the molecular dimensions of miRNA, would result in an inherent signal amplification.

2. Experimental

Thiolated PNA probe (HS-PNA: N′-Thiol-C6-OO-GCTTTTTGCTCGTC TTAT-C′) was from Eurogentec. All natural nucleic acids were custom synthesized and HPLC purified (Sigma-Aldrich); targets: miR-208a microRNA (5′-AUAAGACGAGCAAAAAGCUUGU-3′), its DNA analogue (DNA-208a: 5′-ATAAGACGAGCAAAAAGCTTGT-3′), a 22-mer random RNA as negative control (NC RNA, 5′-AGUACUAAUUCGUCUCUGUUCU-

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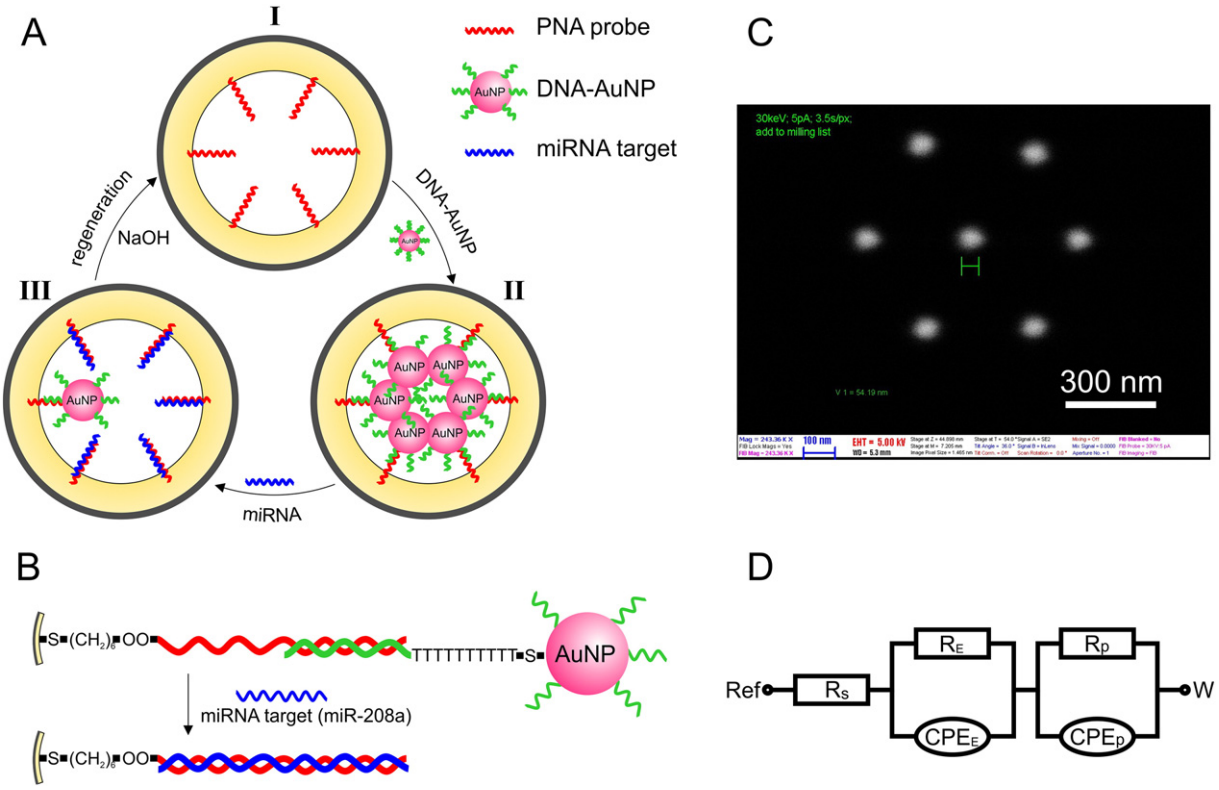

Scheme 1. (A) Schematics of the nanoparticle displacement assay with nanopore sensors for the detection of microRNA. (I) PNA-functionalized nanopore. (II) Nanopores after anchoring DNA-AuNPs by hybridization (corresponds to the ready-to-measure state). (III) The target microRNA by binding to the PNA displaces DNA-AuNPs decreasing the pore resistance. (B) Probe design for displacement assay. (C) Secondary electron yield map of the nanopore arrays. (D) Equivalent circuit for fitting impedance spectra.

3′). For the modification of the AuNPs a short complementary thiolated DNA (HS-DNA) with T_{10} spacer, 5′-Thiol-C6-T_{10}-ATAAGACGAGC-3′) and non-complementary (HS-NC DNA, 5′-Tiol-C6-TTGACCAACAAGCT TTTTTT-3′) DNA strands were used. All solutions were made of highest grade chemicals for molecular biology and with DI water (18.2 MΩ cm) or RNase-free water (Sigma-Aldrich).

2.1. Fabrication of solid-state gold nanopore arrays

Gold nanopore arrays consisting of 7 nanopores (Scheme 1C) were fabricated by combined micromachining and FIB milling as described earlier [28,29]. In essence, slightly conically-shaped nanopores were drilled by FIB in thin silicon nitride (200 nm, SiN_x) supported gold membranes (150 nm, Au). We used nanopores with 40, 60 or 80 nm smallest pore diameters as determined by a secondary electron yield map generated by Ga^+ ions passing through the pore and reaching a metal target behind the membrane (Zeiss Leo 1540 XB SEM, Canion FIB nanoprocessing system, Carl Zeiss).

2.2. Preparation of HS-DNA probe modified gold nanoparticles

HS-DNA probe modified AuNP solution was prepared as described earlier [30]. First, 13 ± 1 nm diameter gold nanoparticles were prepared by reducing $HAuCl_4$ with a citrate salt and determining their concentration by measuring the absorbance at 524 nm ($\varepsilon = 2.7 \times 10^8$ L mol$^{-1}\cdot$cm^{-1}) [31]. The AuNP solution was concentrated tenfold to 120 nM by centrifugation at 16,000*g* for 10 min. Conjugation of AuNPs with HS-DNA probes was performed by adding 21 μL of 100 μM HS-DNA solution to 400 μL of AuNP solution. To increase the DNA probe density on the AuNPs, the ionic strength was increased in three consecutive steps: first 46.8 μL 100 mM NaCl solution was added and vortexed for 30 min, then 19.7 μL of 1 M NaCl and vortexed for another 30 min, and finally 27 μL of 1 M NaCl to reach a final NaCl concentration of 100 mM. DNA-AuNP solution was aged at 4 °C overnight before adding 57 μL aqueous solution of 10 mM (11-mercaptoundecyl)tetra(ethylene glycol) (HS-TEG) and vortexing for 30 min. The unreacted HS-TEG was removed by replacing the supernatant with 1 mM NaCl at least six times after centrifugation at 16,000*g* for 10 min.

2.3. Functionalization of the gold nanopore arrays

Prior to functionalization, the nanopore chips were cleaned with piranha solution for 30 min, thoroughly rinsed with DI water and dried. Gold nanopores were functionalized with HS-PNA probes in a prehybridized form with a complementary DNA to ensure self-regulated surface coverage of PNA on gold by concomitant minimization of the non-specific strand adsorptions [27]. The nanoporous membranes were modified by placing 5 μM miR-208a specific PNA prehybridized with DNA-208a (20 μL) in borate buffered saline onto their surface, covered to avoid evaporation and incubated at 4 °C overnight. The membranes were then rinsed with DI water and blocked with 1 mM HS-TEG solution for one hour. Before use the immobilized PNA layer was activated by dehybridizing the complementary DNA strands using 50 mM NaOH (2 × 1 mL). The optimized procedure to load the DNA-AuNPs into the nanopores implied forcing 5 μM DNA-AuNP solution in 50 mM NaCl into the nanopore membrane by applying 16 kPa pressure difference for 60 min.

2.4. Electrochemical measurements

The resistance of the nanopore membrane was determined by impedance spectroscopy using a Reference 600-Potentiostat (Gamry Instruments) and a custom-made transport cell with the two compartments separated by the nanoporous membrane. Each