CHINESE JOURNAL OF ANALYTICAL CHEMISTRY
Volume 43, Issue 7, July 2015
Online English edition of the Chinese language journal

Available online at www.sciencedirect.com
ScienceDirect

Cite this article as: Chin J Anal Chem, 2015, 43(7), 971–976.

RESEARCH PAPER

A Low Noise Amplifier System for Nanopore-based Single Molecule Analysis

YAN Bing-Yong, GU Zhen, GAO Rui, CAO Chan, YING Yi-Lun, MA Wei, LONG Yi-Tao*

Department of Automation& Key Laboratory for Advanced Materials, East China University of Science and Technology, Shanghai 200237, China

Abstract: A novel amplifier system was designed and prepared for low-noise recording of pico-ampere current in nanopore experiment (< 100 pA). As an example, the amplifier system was applied in α-hemolysin based nanopore detection of DNA-PEG-DNA conjugate to record the signals of translocation and bumping events in buffer solution (1 M KCl, 10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The amplified current signal was filtered by a 3 kHz Bessel filter and sampled by a 100 kHz analog-digital convertor. As a result, the presented amplifier system could lower the noise in recording the current. Current blockages (< 10 pA) of single molecules with low amplitude were recovered due to the high signal-to-noise ratio.

Key Words: Nanopore; Single molecule detection; Current amplifier; Denoising

1 Introduction

In the last two decades, studies based on monitoring the current fluctuation across nanopore received a great deal of attention, due to their promising applications for rapid, sensitive and label-free detection of individual molecules[1–4]. Most of the studies were carried out by using the nanopores for resistive-pulse sensing experiments. Transient current change induced by a single molecule could inform their unique properties which were hardly obtained in ensemble measurements. For example, the sequence of DNA was directly read out as the bases passed through the nanopore one after another, inducing different degrees of current blockages[5,6]. In addition, the versatility of nanopore was demonstrated in the studies such as sensing the structures of RNA[7–9], peptides[10–12] and proteins[13–15], detecting the interactions between supramolecules[16,17], measuring the size of nanoparticles[18,19] and concentrations of metal ions[20].

However, the ionic current across nanopore was extremely low (picoampere to nanoampere) owing to their high resistance. For example, the open pore current of α-hemolysin nanopore was as low as 100 pA at a voltage bias of 100 mV (1 M KCl), and the blockage of current was less than 20 pA at some cases[21]. Therefore, those small current signals could be submerged by the noise in the recording process[22,23]. Though the development in data analysis enables extracting the blockage information from current trace at condition of high noise, the missing of short-lived events is still inevitable[24,25]. Physically suppressing the noise in signal remains a feasible and direct way to improve the performance of nanopore measurement. Conventionally, the current was measured and converted into a voltage signal by using a resistor-feedback amplifier[26]. The resistance of the feedback resistor on the order of 0.1–10 GΩ controls the gain of current, whereas it also contributes to the majority noise in the signal. Though using a large resistance can lower the noise, the dynamic range for current measurement will also be narrowed due to the saturation of amplifier[27]. Other methods for lowering noise have been developed. For example, Rosenstein *et al*[28] fabricated an integrated nanopore sensing platform to improve the signal-to-noise ratio at high bandwidth. Balan *et al*[29] suppressed the current noise by reducing the nanopore chip

Received 2 March 2015; accepted 4 May 2015
* Corresponding author. Email: ytlong@ecust.edu.cn
This work was supported by by the National Natural Science Foundation of China (Nos. 21125522, 21327807, 51407078) and the Fundamental Research Funds for the Central Universities of China (Nos. 222201313004, WH1514049).

DOI: 10.1016/S1872-2040(15)60843-X

capacitance.

In this study, a novel design of the circuit of amplifier system was presented for low-noise current measurement (Fig.1a). The amplifier system features in a capacitor-feedback amplifier (A_1) and an inverting differential integrator (A_3). The advantages of capacitor-feedback amplifier were demonstrated by Goldstein *et al*[30], and the thermal noise on the absence of feedback resistor was effectively reduced. The differential integrator could highly suppress the common-mode noise between the current signal and reference voltage. Furthermore, a drift compensation was introduced for the reference voltage.

2 Experimental

2.1 Materials

α-Hemolysin and decane (≥ 99%) were purchased from Sigma-Aldrich (St.Louis, MO, USA). 1,2-diphytanoly-*sn*-glycero-3-phosphocholine (chloroform, ≥ 99%) was purchased from Avanti Polar Lipids (Alabaster, AL, USA). Ultrapure water (18.2 MΩ·cm at 25°C) was obtained by the Milli-Q System (EMD Millipore, Billerica, MA, USA.). Buffer solution (1 M KCl, 10 mM Tris-HCl, 1 mM EDTA, pH 8.0) was prepared before use. All other chemicals were of analytical grade. The detected DNA-PEG-DNA conjugate (5'-GTCACGATGGCCCAGTAGTT-HPO$_4$-(CH$_2$-CH$_2$-O)$_9$-TTGATGACCCGGTAGCACTG-3') was synthesized and HPLC-purified by Sangon Biotech (Shanghai) Co. Ltd. (China).

2.2 Nanopore experiments

As described previously, 1,2-diphytanoly-*sn*-glycero-3-phosphocholine in decane (30 mg mL^{-1}) was applied to form a bilayer across a 150 μm orifice in a lipid bilayer chamber (Warner Instruments, Hamden, CT, USA) which was filled with buffer solution. The solution of *α*-hemolysin was injected to the *cis* chamber proximal to the bilayer. Then, seven monomers of *α*-hemolysin would assemble to form a hydrophilic channel in the bilayer. The two compartments of the bilayer cell were termed *cis* and *trans* (shown in Fig.1). A pair of Ag/AgCl electrode was inserted into the two compartments, respectively. After a single nanopore was formed on the bilayer, analyte was injected into the *cis* chamber. The voltage was set to +120 mV during the experiments.

The presented amplifier and a commercial resistor-feedback amplifier system (ChemClamp, Dagan Corporation, Minneapolis, MN, USA) were used to amplify and measure the ionic current flowing through the nanopore, respectively. The amplified currents were filtered at 3 kHz and converted to digital signals by DigiData 1440A hardware (Axon Instruments,

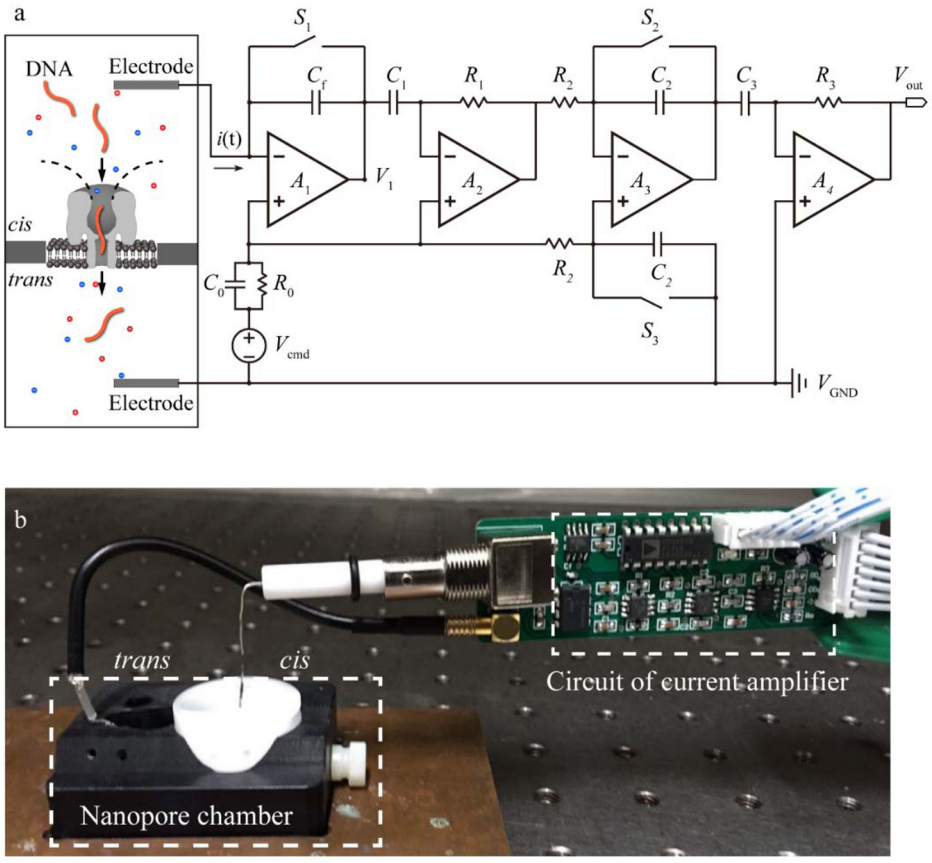

Fig.1 Schematic (a) and photograph (b) of the setup of the nanopore experiment and the amplifier circuit