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A rupture detection algorithm for the DNA translocation detection though biological nanopore

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Abstract

We propose in this paper a new methodology for the treatment of electrical signal of DNA translocation through α -hemolysin biological nanopore formed on an artificial lipidic membrane reconstituted on a microfluidic 96 wells microarray. The signal treatment algorithm is capable to detect translocation events through the pore, within very long and noisy recordings on multiple chambers of our microarray. The firing events are precisely detected, as well as the duration and amplitude of the event, thanks to a rupture detection algorithm. This signal treatment analysis, in association to our artificial bilayer microarray technology, might bring a substantial step beyond highly parallelized biosensing of DNA on a chip.

Keywords: Type your keywords here, separated by semicolons ; DNA translocation, signal processing, nanopore

1. Introduction

In view of the DNA sequencing, we fabricated a biochip dedicated to the DNA translocation through natural nanopores reconstituted on an artificial biomimetic membrane. Translocation events are electrically detected, sampled and treated through a rupture detection algorithm described in this paper. The biochip permits to reconstruct artificial lipid bilayers that are arrayed for the insertion of membrane proteins which can be used once assembled as natural nanopore. The bilayer is facing microfluidic channel and chamber, connected to electrical monitoring wires (figure 1). The fluidic structure of the microarray is achieved thanks to micro-stereolithography and includes a poly(*p*-xylylene) thin film ($t=10\text{ }\mu\text{m}$) that has been etched through oxygen plasma in order to pattern array of holes for the bilayers reconstruction. The microfabrication process is described elsewhere^{1,2}. We use this microarray as a sensor to monitor the DNA translocation through α -hemolysin natural nanopores³ inserted within the biomimetic bilayers. The passage of DNA strand induces a current blockade, measured with patch clamp method, whose amplitude and duration is supposed to characterize the DNA composition and length. Nevertheless the multiplicity and quantity of electrical information requires signal processing, in order to extract the answer to the biological problem which is addressed. In particular the software has to be able to detect the duration of the translocation, as well as its time of apparition, despite high noise in the measured signal. It should be also capable to discriminate the translocation event with other events like translocation tentative, or clogging of the natural pore. Very long recording duration (several minutes at high frequency sampling), leading to large data volume, needs to be analyzed in this way.

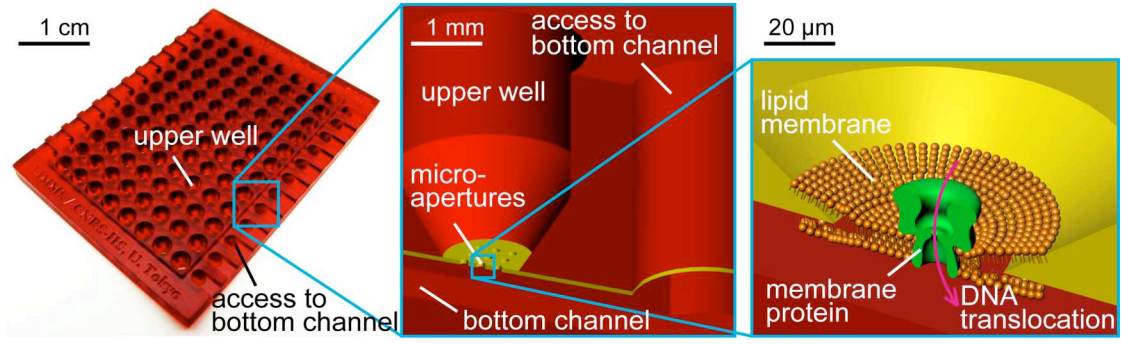


Fig 1: 96 wells biochip for the parallel recording of ion channels

2. The rupture detection algorithm

We propose to apply a “rupture detection” algorithm⁴, represented on figure 2. h_1 and h_2 are normalized door functions that we apply to the variance of the measurement signal $\hat{\mu}(t)$. The rupture is detected, as the variance is proportional to the distance of the signal from its average. h_1 length determines the noise rejection capabilities of our filter, as h_2 determines its capabilities to detect ruptures in the signal. As we can see of figure 3, where a short recording of the measured signal is represented, when we tune h_1 and h_2 of this filter to an appropriate value, the DNA translocation events or clogging events are detected, while the tentative passages are filtered.

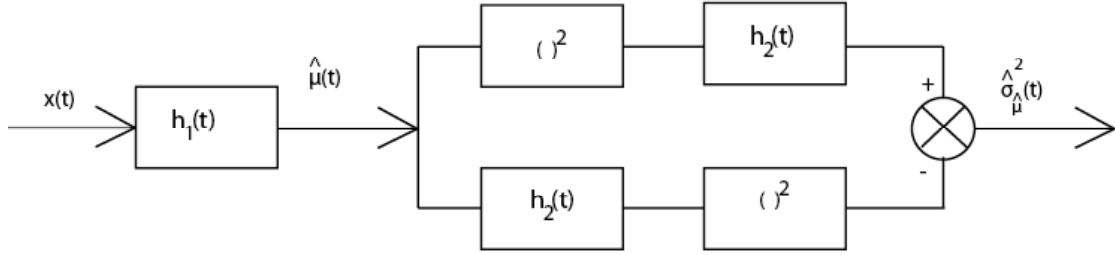


Figure 2: the rupture detection algorithm

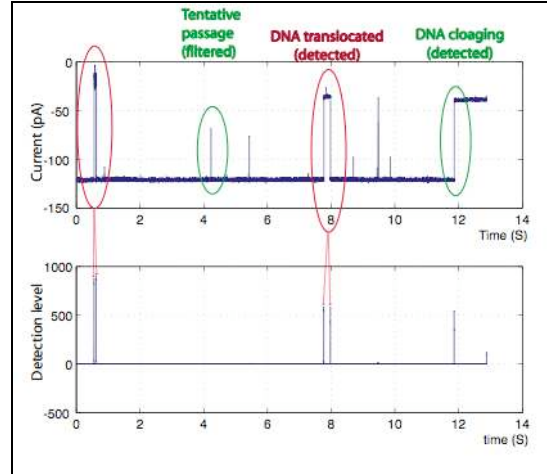


Figure 3: Detection of DNA translocation, filtering tentative passage and DNA clogging

3. Results and discussion

The filter was applied to measure the translocation of 41mer Single Strand DNA: TTTTTTTTCACTGACCTGGGGGAGTATTGCGGAGGAAGGT at 45 μ M concentration in buffer 1M KCl, 10mM PBS, 1mM EDTA, pH 7.4. Once the bilayer was formed thanks to the painting method, and α -hemolysin inserted as described elsewhere,[2], a -80mV continuous voltage was applied between both sides of the biomimetic membrane. Current through α -hemolysin was recorded with patch-clamp recorder CEZ-2400 (Nihon Kodon Co., Japan), at 50kHz sampling frequency and with a 4th order Bessel filter at 10kHz.

As shown on figure 4 an α -hemolysin channel was formed at 194 s. The protein channel was clogged at 207s, 283s, 296s, and 366s, as shown of the figure, and we had to change the voltage polarity (from -80 mV to 40 mV) in order to free the channel.

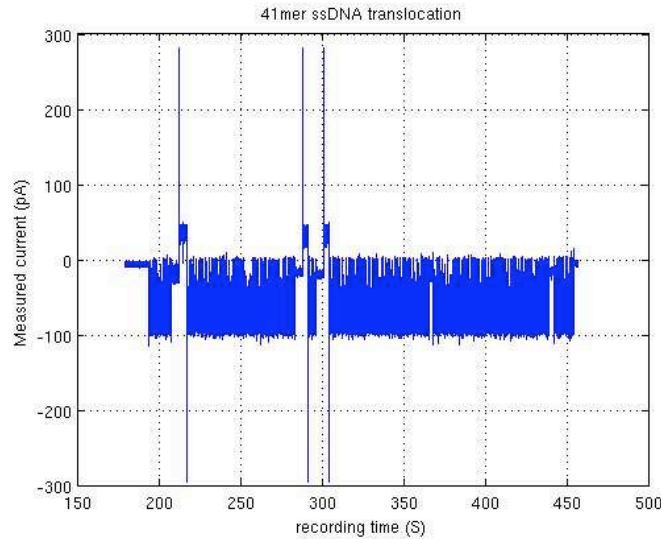


Figure 4: Translocation signal

364 translocation events were recorded during 250s, which makes unavoidable the use of a dedicated software to characterize this signals (duration, amplitude,...). A magnified view of a translocation event is provided on figure 5, on which is superposed the rupture detection output. A delay occurs between the translocation event and the peak of rupture detection output (see figure 5 left). This delay, that depends directly on L1 and L2, can be avoided if the difference is calculated between the output of the rupture algorithm applied for the increasing times, and applied for the decreasing times (see figure 5, right). In that case the rupture event corresponds to the zero crossing of the detector.

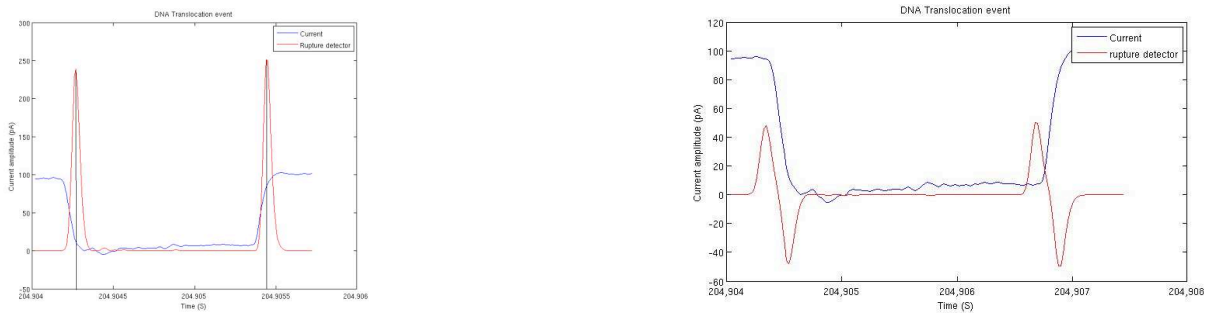


Figure 5: Magnified view of a translocation event. left: the output of the rupture detection algorithm right: differentiation between the rupture algorithm applied for increasing and decreasing times

The distribution of translocation event duration and amplitude was calculated from the result of the algorithm. The result, shown on figure 6 shows that the duration of translocation is centred around 1.2mS, while amplitude of translocation peaks is distributed around 55V and 85V. 55V correspond to incomplete translocations.

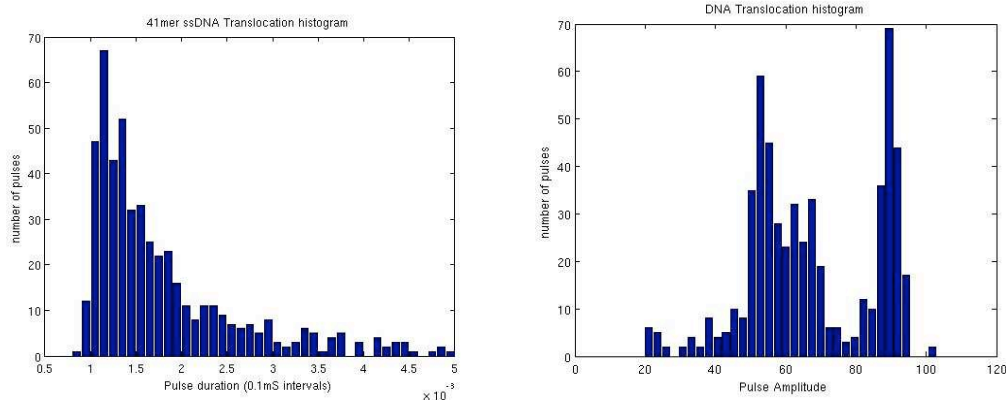


Figure 6: distribution of translocation events Left: distribution of the translocation duration Right: distribution of the translocation amplitude

The rupture detection algorithm shows to be very robust and noise rejecting, and quite appropriate for the analysis of signal issued from the DNA translocation. Next step will be now to make profit of the noise rejection capabilities of the filter, by increasing the sampling and low pass filter frequencies. By doing so we hope to extract the translocating DNA composition.

Acknowledgements

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References

- [1] Suzuki, H., Pioufle, B. L. & Takeuchi, S. Ninety-six-well planar lipid bilayer chip for ion channel recording Fabricated by hybrid stereolithography. *Biomed Microdevices* 11, (2009), 17-22.
- [2] Osaki, T., Suzuki, H., Le Pioufle, B. & Takeuchi, S. Multichannel Simultaneous Measurements of Single-Molecule Translocation in OE \pm -Hemolysin Nanopore Array. *Analytical chemistry* 81, (2009), 9866-9870.
- [3] Song, L. et al. Structure of Staphylococcal alpha -Hemolysin, a Heptameric Transmembrane Pore. *Science* 274, (1996), 1859-1865.
- [4] Liu, W., Magnin, I. & Gimenez, G. Opérateur pour la détection de rupture dans des signaux bruités. *Traitement du signal* 12, (1995).