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Supporting Material

DNA translocation and unzipping through a nanopore: some geometrical effects.

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1 Supplementary Information

1.1 Cluster Boundaries definitions

We do not have a perfectly rigorous way of defining the clustering of the events. The boundaries of the clusters are fixed in a somewhat arbitrary way that however fulfill the following criteria. In the forward direction, the selected clusters (ellipses on figure ??A), are defined by the following criteria: the selected traces should present a steady blocked current level below 20%. The observed noise should not be abnormally high (above 2.5pA rms). The cluster boundaries should also correspond to the visual impression of data clustering. The 2 clusters represented on figure 2A include 90% of the 5700 events in the forward direction. The distinction between the 3' and 5' cluster is mainly based on the blocked current value although the already reported overlap between the 3' and 5' event prevents to unambiguously attribute a DNA orientation for some events. Our quantitative results are changed only by a few percent if all the translocations below 10^{-3} seconds are taken into account.

In the backward direction, the clustering was harder to achieve. The main cluster coined 3' is defined with the criteria mentioned above. Following the interpretation that we gave for the long events (above 10^{-3} s) we additionally excluded them from the analysis. Notice that these events also exist in the forward direction in a much smaller amount and are usually withdrawn from analysis. The second cluster called 5' is probably the most arbitrary of all four. It corresponds to events with steady traces of low blocked current within the limit of times expected for a translocation. The selected events represent 62% of the 4100 traces recorded in the backward direction. Hence defined, these four clusters shift to smaller times when the voltage is increased, whereas the rest of the events are nearly voltage insensitive. We thus believe that this clustering capture the main feature of the translocation process. Changing the boundaries of the cluster by 10% for the blocked current and 20% for the time marginally modifies our quantitative results.

1.2 Experimental setup

All translocation recordings through alpha-hemolysin were performed on a set-up previously described in [21]. Briefly, a single alpha-hemolysin nanopore was incorporated in a lipid bilayer of about $30 \mu m$ in diameter formed in a teflon septum. The DNA strands were always added to the electrically grounded cis chamber. A positive voltage is applied across the bilayer. The alpha hemolysin monomers were added to the cis chamber and the bilayer is reformed until a single pore is steadily incorporated with the desired orientation characterized by the asymmetry of its I-V curve. Before DNA was added, we verified that the pore was stable for 5 minutes. The ionic current signal was recorded using a Axon Axopatch 200B via a National Instrument acquisition card. For ssDNA experiments we used a sampling rate of 1 MHz with the full bandwidth of the amplifier. For experiments on hairpins the sampling rate was reduced to 100 kHz and the signal filtered by a 4 pole Bessel filter at 10 kHz. Each event was analyzed using IgorPro (Wavemetrics), extracting the mean open pore current, the mean blocked pore current, the event duration and inter-event time using a standard thresholding method (event threshold fixed at 70% of the open pore current).