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Detection of DNA hybridizations using solid-state nanopores

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Abstract

We report an experimental study of using DNA translocation through solid-state nanopores to detect the sequential arrangement of two double-stranded 12-mer hybridization segments on a single-stranded DNA molecule. The sample DNA is a trimer molecule formed by hybridizing three single-stranded oligonucleotides. A polystyrene bead is attached to the end of the trimer DNA, providing a mechanism in slowing down the translocation and suppressing the thermal diffusion, thereby allowing the detection of short features of DNA by standard patch-clamp electronics. The electrical signature of the translocation of a trimer molecule through a nanopore has been identified successfully in the temporal traces of ionic current. The results reported here represent the first successful attempt in using a solid-state nanopore as an ionic scanning device in resolving individual hybridization segments (or 'probes') on a DNA molecule.

1. Introduction

Voltage-driven translocation of biopolymers through nanometer-scale α -hemolysin pores was first reported by Kasianowicz *et al* [1] in 1996, and it has been shown [2, 3] to be an effective process for dynamically linearizing DNA and RNA molecules in buffer conditions. Significant theoretical advances have been made [4–6] in understanding the roles of entropy and the DNA-pore interactions in the DNA translocation process. There is also growing interest in exploiting this linear transport process for applications in biotechnology such as DNA sequencing [7] and molecular diagnostics. It has been demonstrated that the voltage-driven translocation process combined with the nanometer-scale aperture of the α -hemolysin pore can be used for unzipping DNA or RNA hairpins [8, 9]. The development of solid-state nanopores [10, 11] has opened up further opportunities in combining physical tools with molecular biology, for example, optical and magnetic tweezers have been successfully used [12, 13] for controlling the DNA translocation process in solid-state nanopores. There are promising experimental results suggesting that single- and double-stranded RNA molecules can be distinguished with solid-state nanopores [14] and the binding of proteins or peptide nucleic acids (PNA) can cause changes in the translocation behavior [15].

A successful quest for a nanopore DNA sequencing technology hinges on solving the issue of sequential detection of nanometer-scale features on a DNA molecule as it is driven

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through a nanopore. One question of current interest is whether it is possible to measure the structural information of a DNA molecule during its transit through a nanopore, e.g. by monitoring the change in ionic current through the pore as envisioned [1] by Kasianowicz *et al.* Given the spatial distance between the nucleotides at 0.4 nm, it is unlikely that one can resolve features on a DNA molecule at single-nucleotide resolution using solid-state nanopores with a minimum channel length of about 10–20 nm. Nanocapillaries that can be made by a relatively simple process of laser-assisted pipette pullers have also been used for sensing biomolecules like DNA [16, 17] and colloidal particles with or without DNA coating [18, 19]. While their diameters can be made to reach the nm range with a simple pipette puller, it would be interesting to see the smallest thickness that can be achieved in order to increase their resolution for DNA sequencing applications.

Nevertheless, it is interesting to experimentally test if it is possible to resolve the intermediate spatial structures, e.g. 10–100 nucleotides, on a single DNA molecule in the temporal profile of the ionic current through a nanopore during DNA translocation. With such a capability, it is conceivable to decipher the sequence of DNA by using the hybridization-assisted nanopore sequencing (HANS) approach [20]. In this approach, short oligomeric DNA probes are bound to a long single-strand target DNA molecule by Watson-Crick base-pairing. By using a library of such short DNA probes and by aligning their ionic current signals, the sequence information of the target DNA can be reconstructed [20]. An important issue is whether a nanopore sensor can resolve short DNA probes at room temperature against thermal fluctuations. Here we report the first electrical detection of 12-base DNA oligomeric probes on single-molecule DNAs with a solid-state nanopore.

2. Experimental details

2.1. Concept of the experiment and DNA sample design

In this experiment, we test the feasibility of using a DNA translocation experiment to resolve two hybridization segments on DNA. Our model DNA system consists of three single-stranded poly-T (thymine) oligonucleotides: 3'-TGTGTGCTGTCG-(T---T)₁₃₀-(Biotin)-5'; 3'-CGACAGCACACA-(T---T)₁₂₀-CGGCTATGTTGG-5' and 3-(T---T)₁₃₀-CCAACATAGCCG-5'. The three single-stranded (ss) oligonucleotide molecules are joined by Watson-Crick base-pairing at the sticky ends to form a trimer structure, ss-ds-ss-ds-ss, with alternating single- and double-stranded (ds) segments (see figure 1(a)). In order for these ssDNA molecules to form a trimer complex, the single strands, each 142 bases long, that form the ends of this complex have only one sticky end whereas the middle strand, 144 bases long, has two sticky ends. Each end of the middle molecule is complementary to the sticky end of only one of the other two strands (figure 1(a)).

In designing the model DNA with desired stable hybridization segments, we used computational biology software from DINAMelt server (<http://dinamelt.bioinfo.rpi.edu/>) to calculate the melting temperature of various sequences. The calculations were done for 1 M Na⁺ ion concentration. For the optimized sequences, base-pairings of single-strand-1 (ss1) with ss2, and ss2 with ss3, show a melting point near or above 60 °C, others being either near or well below room temperature (table 1, supplementary materials available at stacks.iop.org/Nano/21/335102/mmedia). Therefore, these ss oligomers are expected to form

stable ss dimer and trimer complexes at room temperature. The formation of dimers and trimers were confirmed using gel electrophoresis which shows the presence of three distinct bands corresponding to monomers, dimers and trimers (figure 1(b)). The stability of the DNA complexes was established by UV absorption measurements at 260 nm in 1 M SPSC buffer from 25 to 70 °C. The melting transitions of the ss1–ss2 and ss2–ss3 DNA segments were identified at temperatures close to 51 °C and 57 °C, respectively [21, 22]. The measured melting point is closer for the ss2–ss3 double-stranded segment to the computationally obtained value of 60.8 °C while it is somewhat lower for the ss1–ss2 segment, 64.2°C (table 1, supplementary materials available at stacks.iop.org/Nano/21/335102/mmedia).

In our model DNA, the trimers have two adjacent ds segments each 12 bases long, or ~4 nm in length, and separated by an ss segment of about 49 nm in contour length. Since the optimum thickness of our nanopore channel length (determined by membrane thickness and TEM-drilling profile) is ~20 nm, we expect that, in principle, when the trimer DNA complex is stretched, the second ds segment will be located well away from the cis side of the nanopore when the first ds segment leaves the trans side of the pore (figure 1(c)).

To reduce the demand in temporal resolution in the detection electronics, we slow down the DNA translocation by attaching a polystyrene bead at one end of the DNA. The average diameter of the beads is ~130 nm, much larger than the pore diameter, and thus we expect the bead to be detached when the DNA is completely pulled through the pore under the large electric field. Figure 1(d) shows a typical trimer translocation event which we identify (with details to be described below) as the signature of trimer DNA translocation through a nanopore. During the DNA translocation, the DNA is under tension (which often causes undesired premature breakage of the streptavidin-biotin bond, see below). Thus the relatively massive bead should help reduce the effects of thermal diffusion of the DNA in the nanopore.

2.2. Oligonucleotide synthesis and characterization

The oligonucleotides, synthesized single strands of DNA molecules and PAGE-purified, were purchased from Midland Certified Reagent Co., Midland, TX, USA. The polymer bead is attached to the biotin linker at the 5' end of one of the oligomers. Their stock solutions were stored at concentrations of 25.1, 1.8 and 9.9 µM for ss1, ss2 and ss3, respectively, in TE (10 mM tris, 1 mM EDTA, pH 8.0) buffer. For forming DNA complexes, the ss DNA stock solutions were mixed and diluted in equal molar concentrations with SPSC buffer (1 M NaCl, 50 mM Na₂HPO₄) to a final optimal concentration of 288 nM.

For electrophoresis runs, gels made from 1.8% of Ultrapure Agarose 1000 (Invitrogen) in 1X LB (dilithium borate–boric acid) buffer from Faster Better Media, LLC were used. The reference is a 50 bp DNA ladder (Invitrogen) and the sample is 20 µl total at 144 nM concentration, with the LB loading buffer. A number of gel electrophoresis runs at different voltage levels and durations were performed to find the optimal conditions. The gel picture shown in figure 1(b) was run for 25 min at 25 V and then 200 min at 75 V. After the voltage was turned off, the gel was placed in SYBR gold stain (Invitrogen) for 40 min, and then photographed with an Olympus C-7070 digital camera with an SYBR photographic filter.

We have found that, in order to see the trimer and dimer bands clearly, it is necessary to place the entire gel columns in an ice bath during the electrophoresis.

The solution containing the mixture of ss DNA molecules was left at least for 14 h overnight to allow the formation of dimer and trimer DNA complexes as seen in the gel electrophoresis patterns. This solution was used to bind them with streptavidin-coated polystyrene beads of 130 nm nominal diameter (ProActive[®] Microspheres, Bangs Laboratories, Inc.) as follows. The beads were first vortexed to ensure homogenization of the stock solution. The aliquot of beads was then washed twice by adding an equal amount of wash buffer (1 M NaCl, 20 mM Tris pH 7.5, 1 mM EDTA, 0.0005% Triton-X 100) and centrifuging at 10 000 rpm for 3–5 min, until the supernatant appeared clear. After decanting, we then added the solution containing the DNA complexes and an amount of wash buffer to bring the concentration to the desired level for mixing. The mixture was left at room temperature in the vortexer at low speed for the desired length of time (either 20 or 120 min). The mixture was then washed, centrifuged and decanted once more as previously described, first with the wash buffer, and then once in the buffer the mixture would finally be stored in. After this final washing, storage buffer, usually SPSC buffer, was added to bring the mixture to the desired final concentration.

2.3. Nanopore DNA translocation experiments

Our solid-state nanopore devices were fabricated using the TEM-drilling method [11]. The pores were drilled in a free-standing silicon nitride membrane (20 nm in thickness) using a focused electron beam in a TEM (JEOL-2010F). The nanopore experimental set-up is similar to the ones used previously [13]. The ionic buffer has a pH of 8.0 with 1 M KCl–10 mM tris–1 mM EDTA (TE) concentration. The measured nanopore resistance was close to the calculated value with the pore dimensions, 10.0 nm × 7.8 nm for nanopore-A and 15.5 nm × 7.3 nm for nanopore-B, obtained from TEM. The open-pore currents show linear I – V characteristics typical of a properly wet and cleaned solid-state nanopore. The power spectral density of the nanopore current shows a characteristic $1/f$ behavior in the low frequency (<5 kHz) region. The current through the nanopore was recorded by an Axopatch 200B voltage-clamp measurement set-up connected to a digitizer through a computer in voltage-clamp mode at fixed applied voltages of 85 and 100 mV. The data was collected at a sampling interval of either 6.5 μ s corresponding to a bandwidth of 153.85 kHz or at 8.0 μ s with a bandwidth of 125 kHz in the different nanopore experiments. The data was passed through a low-pass, four-pole Bessel filter with a cutoff frequency of 10 kHz before digitization.

3. Results

3.1. DNA translocation experiments with nanopore-A

After filling the cis side of the nanopore with the DNA-bead complex, the nanopore current was recorded at 85 mV for about 30 min until the DNA-bead complexes reach the nanopore and then the voltage was increased to 100 mV, at which point in time an avalanche of events were detected (figure 2(a)). The nanopore current histogram recorded before the onset of the avalanche of events shows a single peak (figure 2(b), black) that can be fitted well by curve-

fitting to a Gaussian distribution (mean = 7.458 ± 0.0001 nA and standard deviation $\sigma = 21.6 \pm 0.1$ pA) which is expected for a stable nanopore. At the onset of the avalanche of translocations, the nanopore current jumps down to ~ 7.2 nA.

Closer examination of this current jump shows two consecutive events as a result of the entry of a folded single-stranded DNA followed by a trimer DNA complex. However the folded single-stranded DNA gets stuck to the nanopore till the end of the avalanche of events resulting in the large peak in the current histogram at ~ 7.2 nA during the avalanche. A similar phenomenon has been observed before in DNA translocation experiments with solid-state nanopores. The histogram of the current distribution during the avalanche shows three major peaks (figure S5-b available at stacks.iop.org/Nano/21/335102/mmedia, gray) and three minor peaks (inset, figure S5-b available at stacks.iop.org/Nano/21/335102/mmedia). However, the examination of the individual translocation events shows that there is an additional major peak that is not resolved in this plot. These seven peaks can be curve-fitted well to a Gaussian distribution each with the peak values (in nanoamperes): 7.218 ± 0.001 , 7.054 ± 0.002 , 6.892 ± 0.012 , 6.784 ± 0.019 , 6.439 ± 0.069 , 6.257 ± 0.063 and 6.025 ± 0.072 . The most dominant peak at 7.218 ± 0.001 nA corresponds to the base current during the avalanche of events as can be seen from the individual events (figures 2(a) and (b) in the main paper). The major curve-fitted peaks correspond to the current drops of 160, 326 and 434 pA. The current drops of 160 and 434 pA agree very well with those calculated for the translocation of a single- and double-stranded DNA of diameters 1.29 nm and 2.12 nm, respectively, for a membrane thickness of 10.65 nm. These diameters are in close agreement with those obtained earlier [23, 24] in nanopore translocation studies. The current drop of 326 pA, being almost double 160 pA, arises from a folded single-stranded DNA entering the nanopore.

3.2. Identifying trimer translocation events with assistance of computer simulation

After a quick scan of the massive amount of translocation events in figure 2(a), one quickly realizes that a computer simulation of trimer DNA translocation is needed to help guide the event analysis task. For example, we show two individual events that appear nearly identical in figures 3(a) and (b). At the onset of these events, the nanopore current shows a sudden drop to about 6.7 nA spanning less than 100 μ s, then rises sharply to an intermediate level close to 7.05 nA before returning nearly back to the open-pore (base) current. Note that the initial sudden drop to 6.7 nA from the base current of ~ 7.2 nA is closer to the current drop, 434 pA, expected for the ds DNA segment and the intermediate current level, 7.05 nA, is closer to the 160 pA expected from an ss unfolded DNA, taking into account the peak-to-peak current noise, 130 pA ($\approx 6\sigma$). Therefore, such a current profile is consistent with what can be expected when the trimer complex attached to the bead translocates through the nanopore with its free end first entering the nanopore (sharp initial current drop), followed by the ss DNA fragment attached to the bead plugging the nanopore (intermediate current level) before breaking off from the bead eventually.

We simulated the translocation of toy models of the designed ss trimer complexes (see supplementary materials section 2.1 available at stacks.iop.org/Nano/21/335102/mmedia for more details). These models have alternating ss and ds DNA segments represented by ss1–

ds1–ss2–ds2–ss3. The DNA segment is modeled as a solid cylinder of uniform cross section with appropriate diameters corresponding to ss and ds DNA. Since nanopores behave as simple ohmic resistors, the ionic current density is given by Ohm's law, $J = \sigma E$, where J is the current density of the nanopore, σ is the conductivity of the ionic buffer solution which is partitioned by the nanopore and E is the electric field applied across the nanopore. This

equation gives the current, $I = \frac{\sigma VA}{t}$, where V is the voltage applied across the nanopore, t is the thickness of the membrane and A is the area of the nanopore. Therefore, when the DNA translocates through a nanopore partially blocking it, the ionic current passing through it is expected to drop, depending on the nanopore area blocked by the different segments of the DNA complex. Since the ss DNA and ds DNA differ by ~1 nm in their diameter and the current blockage during their translocation depends on the area of the nanopore they block, they can be distinguished with a nanopore, as has been demonstrated for the RNA and DNA molecules [14]. Therefore the ss DNA being smaller in diameter would give rise to smaller current blockage than a ds DNA as distinct levels in the current profile as shown in figure 3 (arrows indicate the ss DNA current blockage level).

The nanopore current was calculated when such a model DNA complex goes through a circular model nanopore (diameter 8.83 nm) with the same cross section and thickness (10.65 nm) as nanopore-A used in the experiment setting the conductivity of the medium to that of the 1 M KCl buffer solution. The diameters of the ds and ss DNA used were 2.12 and 1.29 nm, obtained from the measured nanopore current drops. Since the contour length of an ss DNA is 49 nm, the lengths of the different segments of the simulated DNA complex ss1–ds1–ss2–ds2–ss3 were taken to be 45–15–5–15–45 nm. Initially the whole DNA complex is considered to be translocating at a constant velocity, $0.90 \text{ nm } \mu\text{s}^{-1}$, obtained from the average time elapsed, 55 μs , arrived at from the fits to the nanopore current drops due to the translocation of two consecutive ds DNA regions (figure 3(a)). The translocation velocity was calculated from the average time elapsed using the average nearest-neighbor distance for the bases in the DNA, 0.34 nm. However, because the experimentally detected events do not show the intermediate current level due to the initial entry of the ssDNA, the simulated events (figures 3(c) and (d)) have the initially entering ss DNA moving twice as fast (see supplementary materials for illustrations of this effect, figure S1 available at stacks.iop.org/Nano/21/335102/mmedia). The simulated current trace which has added white Gaussian noise (figure 3(c)) was passed through a simulated low-pass Bessel filter with a cutoff frequency of 10 kHz (figure 3(d)). The filtered current trace looks very similar to the experimentally detected ones (figures 3(a) and (b)). The two dips in the initial large current drop region (~6.8 nA) that correspond to the passage of consecutive ds segments and the intermediate current level arising from the ss DNA plugging the nanopore in the simulated events are quite close to those in the experimentally detected events. This close similarity confirms that these events arise from the trimer DNA complex translocating through the nanopore. (See supplementary materials section 2.1 available at stacks.iop.org/Nano/21/335102/mmedia for details.)

3.3. Resolving hybridization segments on individual single-molecule DNAs in translocation

There are two types of events that we attribute to trimer translocations. The most frequent type of trimer events are shown in figure 4(a). These events only show the initial sudden large current drop with two dips but without the following intermediate current level due to the bead-attached ss DNA plugging the nanopore. These events show two current dips between 400 and 500 pA lower than the base current (figure 4(a)). The time span of these current drops are also comparable (figure 4(b)).

The histogram of the current drops shows a peak that can be fitted to a Gaussian centered at -385 pA (figure 4(c)) which is closer to the calculated current drop of 434 pA for a ds DNA segment of diameter 2.12 nm, considering the peak-to-peak current noise ~ 130 pA ($\approx 6\sigma$). Again, the diameter of the ds DNA used is in close agreement with those obtained earlier [23, 24]. After these checks, the identified current traces were then fitted with two inverted Gaussians yielding the widths of the dips and the separation between them, shown in figure 4.

The peak width is $\sim 54 \pm 1$ μ s which is almost half the period of the Bessel filter cutoff frequency, 10 kHz, used in the experiment. This shows that the measured average peak width is largely determined by the filter cutoff frequency. The average time elapsed, 72 ± 2 μ s, between the translocations of the two consecutive ds DNA segments was obtained from the fit to the histogram of the peak separation with a double exponential (figure 4(d)). The error in the fit is much smaller than the sampling time interval of 6.5 μ s used in the experiment. Thus we are confident to conclude that the data in figure 4(d) is evidence that we have resolved the spatial separation of the two hybridized segments of a single-molecule DNA in a translocation experiment. To demonstrate that we can detect similar types of events from the DNA complexes independent of the nanopore used, we present the analysis of the translocation experiments with another solid-state nanopore (B) which shows similar results as summarized in the supplementary materials along with the discussion of the simulated events, see figures S3 and S2 available at stacks.iop.org/Nano/21/335102/mmedia in sections 3 and 2.2.

Figure 5 shows a second type of rare translocation events. These three events, which have been aligned at the leading edge and averaged to reduce the effect of the fluctuations due to the nanopore current noise on the translocation current profile, are replotted in figure 5(b). The averaged nanopore current is fitted to a set of two inverted Gaussians after taking into account the asymmetric current background with suitable sigmoid-like functions (figure 5(c)). As can be seen from the figure, the Gaussians fit the current variation during the translocation of the ds regions of the trimer complex through the nanopore very well. The best fit obtained by curve-fitting yields a full width of 77 ± 3 and 69 ± 3 μ s for the two current dips from the two consecutive ds regions of the trimer DNA complex with the separation between them, 89 ± 3 μ s. Again, it is clear that we have resolved in temporal trace of the ionic current the spatial separation between the two hybridization segments, even though our time resolution is not good enough to resolve the details of the individual segments.

The current drops from these events appear very similar to the simulations of the translocation of a toy model that has the same type of alternating segments of ss and ds DNA, ss1–ds1–ss2–ds2–ss3, like the trimer DNA complex (see supplementary materials figure S1 available at stacks.iop.org/Nano/21/335102/mmedia). Note that, again, the error in the fits is smaller than the sampling time interval of 8.0 μ s.

The average time taken by a single base to translocate the nanopore is calculated to be 0.64 μ s from the average time duration for the two consecutive ds regions to pass through the nanopore, 89 μ s, obtained from curve-fitting. This is comparable to that obtained from the trimer DNA complex events (figures 3(a) and 4(d)) discussed earlier. This translocation time is more than 34 times faster than for polyadenylic acid RNA molecules, 22 ± 6 μ s per nucleotide (nt), translocating through a biological nanopore α -hemolysin [3] and about 16 times slower than for λ -DNA, 40 ns/nt, measured with solid-state nanopores [23]. Therefore it is clear from the discussion of both types of trimer DNA complex translocation events that the ds regions of the trimer complexes translocate significantly slower than the λ -DNA in solid-state nanopores and faster than poly-A in α -hemolysin, which is known to be much slower due to the strong polymer-nanopore interaction as a result of the narrow constriction in the latter. This clearly shows that the polystyrene bead attached to the trimer DNA complex slows down the translocation and enables the identification of the ds segments formed by 12 bases in our custom-designed DNA complexes which would not otherwise be possible to resolve with solid-state nanopores.

3.4. Bead-DNA separation during translocation: breakage of streptavidin-biotin bond

We attribute the tension generated by the DNA complex being pulled by the electric potential gradient across the nanopore as the cause for the breakage of the streptavidin-biotin bond. It has been found that the streptavidin-biotin bond can withstand a force of 120 pN for a loading rate of 200 pN s⁻¹ by AFM studies [25]. However, for smaller loading rates of 10 pN s⁻¹, it can withstand only about 40 pN measured by biomembrane force spectroscopy [26]. The force on the DNA translocating through the nanopore has also been measured [12] by optical tweezers to be 24 pN for 100 mV. The most probable rupture force for a loading rate of 7.7 pN s⁻¹ has been measured to be between 3.6 and 5.4 pN with optical tweezers [27]. In the light of these measurements of rupture forces for different loading rates reported in the literature, it is not surprising that streptavidin-biotin bond breakage events are seen in our experiment. The drag force experienced by a 130 nm bead moving at the same velocity as the DNA during translocation is calculated to be 13 pN. This force is about half that acting on a λ -DNA translocating through the nanopore at 100 mV, measured by optical tweezers [12]. However, since the streptavidin-biotin bond can break at a rupture force as small as 3.6 pN for small loading rates and the loading rate in the nanopore experiment could have a range of values depending on how far the bead is from the nanopore when the DNA translocates, the observation of different types of DNA complex events, that either show the intermediate current level due to the ss DNA plugging the nanopore or its absence, is not inconsistent with these known rupture forces for streptavidin-biotin bonds.

3.5. Bead as a slowing-down mechanism for DNA translocation

Here it is worthwhile to comment on the use of polystyrene beads as a slowing-down mechanism for DNA translocation. Far from the nanopore where the electric field would be negligible, the effect of impulsive random forces due to the thermal bath on the bead would be damped over a characteristic time, $t_d = m/\gamma$, where m is the mass of the particle and γ is the damping coefficient of the fluid which is related to the drag force that can be calculated from Stokes' law [28]. For a 130 nm spherical polystyrene bead, assuming it has the same density as water, it turns out to be $\sim$ ns, while for a 1 μ m bead it is $\sim$ μ s. Therefore over the duration of $\sim$ 100 μ s during which the ds segments of the trimer complex translocate, the bead can be considered to be stationary. However, during translocation, if the center of mass of the bead is located at a distance longer than the contour length of the DNA complex, the bead will be pulled by the DNA which is acted upon by the electric potential gradient across the nanopore. In such a scenario, in order for it to translocate through the nanopore, it will have to pull the bead at its translocation speed. The drag force on the bead at this speed is calculated to be 13 pN using Stokes' law, $F_{\text{drag}} = 6\pi\eta r v$, where r is the radius of the sphere, v is the speed of the bead and η is the viscosity of the medium. Since the drag force depends directly on the size of the bead, if one increases the bead size by a factor of two, one could expect significant enhancement in slowing down of the DNA translocation speed. In this study, we chose to use polystyrene beads due to their high degree of size uniformity and also the ease of availability of streptavidin-coated beads.

Finally, it is worth pointing out that, among the avalanche of translocation events, the most frequent events are those due to dimer and monomer DNA complex translocation events. (The data are shown in the supplementary materials section 4 available at stacks.iop.org/Nano/21/335102/mmedia.) This is not surprising since, in equilibrium, each bead can have monomer, dimer and trimer DNAs on its surface. In fact, the gel electrophoresis data in figure 1(b) already indicated that trimers are the rarest.

4. Conclusions

The experiments we have described here using solid-state nanopores demonstrate the detection of translocation events from different complexes of ss DNA molecules and, in particular, the successful detection of two consecutive 12-base-long ds segments formed by a trimer complex. Further, these ds segments are separated by only about 140 bases. This is the first experimental demonstration of the electrical detection of very short DNA hybridization probes. A recent nanopore study [15] reported the preliminary evidence of the effects of short PNA tags attached to a ds DNA molecule on the ionic current profile of translocation events. Our result here is the first systematic study of the feasibility of using the temporal profile of ionic current for resolving the spatial separation of two hybridization segments on single molecules of trimer DNAs. Our study shows that these nanoscale DNA hybridization segments (or probes) can be detected by nanopores at room temperature. Our results provide encouragement for the HANS method in sequencing long DNA molecules using nanopores and hybridization probes. The fact that the temporal separation between the two dips has a wide distribution implies that thermal diffusion of DNA in the nanopore is

playing a significant role. Future research will need to address this issue, for example by using larger beads.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

The structure of the designed model DNA molecule formed by hybridization between three oligonucleotides. The middle single-stranded segment (between two encircled regions) of poly-T (thymine) has different 12-base-long sequences on each end that are complementary to the same number of bases on one end of the other two strands (to the left and right of encircled regions). This facilitates the formation of two double-strand regions by Watson–Crick base-pairing scheme when these single strands are mixed under suitable conditions (described in section 2). The sequence of bases in the double-strand regions is shown in the inset. One of the single strands (left) is connected to a biotin molecule at the free end that is used to attach the complex to a streptavidin-coated polystyrene bead through biotin–streptavidin binding. (b) The gel electrophoresis pattern obtained when running the solutions of mixtures containing one, two and three designed single-stranded DNA molecules under

an applied voltage of 25 V (25 min) and then 75 V (200 min) with a 50 bp DNA ladder reference. Lanes L and A refer to the 50 bp DNA ladder and the ssDNA complex samples. Bands labeled m, d and t are formed by the monomers, dimers and trimers of the ss DNA. (c) The schematic picture of the ssDNA trimer complex that translocates through the nanopore. For clarity, the length of the single strands, the bead diameter and the thickness of the pore have been drawn to scale to illustrate that when the first double-strand segment (regions where the two thick lines representing single-strand segments meet) exits the nanopore, the second double-strand segment is still well away from the nanopore. The bead is not drawn to scale, however. This should facilitate the resolution of the double-stranded segments in the DNA complex ionic current blockades recorded in the experiment. (d) An example of a trimer complex translocation event recorded in a nanopore experiment. The black arrows point out the positions of the current drops due to the two consecutive double-strand segments in the trimer DNA complex.

Figure 2.

(a) The nanopore ionic current trace before and after the avalanche of events are detected at 100 mV with nanopore-A, 10.0 nm $\times$ 7.8 nm, after adding the bead-attached single-stranded DNA complexes. (b) The histograms of the nanopore current trace shown in (a), before (black) and after the detection of the avalanche of events (gray) fitted to Gaussian distributions. The fitted value for the current peak before the onset of translocations is 7.458 ± 0.0001 nA. The current distribution for the avalanche of translocation events shows three prominent, one unresolved and three very minor peaks (inset), which can all be fitted well to Gaussians centered near the peaks.

Figure 3.

Two similar trimer DNA complex translocation events are shown in (a) and (b). For comparison, the translocation events simulated from a hard rod model of trimer DNA complex consisting of alternating single- and double-stranded DNA regions (ss-ds-ss-ds-ss) are shown in (c) and (d) (see text). An effective circular nanopore corresponding to the size of the nanopore used in the experiment with effective membrane thickness of 10.65 nm and the conductivity of 1 M KCl ionic buffer solution were used in the simulations. A white Gaussian noise of peak-to-peak value of 130 pA ($\approx 6\sigma$) measured in the nanopore experiment was added to the simulated current from the rigid rod trimer DNA complex model (c) and then filtered with a Bessel filter with 10 kHz cutoff frequency (d) as used in the nanopore experiment to compare with the experimentally detected events. The arrow points to the single-strand current blockade level and the dotted line represents the moment at which the double-stranded DNA segment passes through the nanopore. The simulations were done with Matlab programming and signal processing tools.

Figure 4.

A sample of translocation events that occur when the trimer complex enters the nanopore and breaks off from the bead either during the translocation process or immediately after (nanopore-A experiment). (b) These events aligned at their leading edge showing a distribution in peak widths and their separations. (c) The histogram of the current drop distribution for these events (52 of them). The distribution of peak separation between two consecutive double-strand segments (d) obtained from the fitted peak widths of the double-strand segments of the trimer complex translocation events. The peak widths and positions

were obtained by curve-fitting to two inverted Gaussians for these events. The solid line is a best fit to a double exponential while the dashed line is a best fit to a Gaussian. The peak width histogram can also be fitted similarly (e).

Figure 5.

(a) Three selected near-identical events from a trimer complex attached to the bead are shown from the nanopore-B experiment. These events aligned at their leading edge and zoomed into the region where two double-strand parts of the DNA trimer complex pass through the nanopore ((b), solid lines) are shown along with the average (dashed line). (c) The best-fit profile of the average (black) is obtained from curve-fitting to two inverted Gaussians (gray in print, red online) with appropriate background.