

Chemical Tags Facilitate the Sensing of Individual DNA Strands with Nanopores**

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Nanopore recording is an electrical analytical technique in which individual molecules block a nanometer-scale pore and cause detectable modulations in ionic current.^[1–5] The single-molecule approach has been exploited to analyze proteins, toxins, metal ions, drug molecules, and single-point mutations in double-stranded DNA.^[6–11] Despite progress in the sensing of isolated nucleotides^[12] and single base positions in static DNA strands,^[13] it has, so far, not been possible to detect multiple bases in an individual strand. One of the main technical hurdles towards this aim is the high speed at which the voltage-driven DNA strands pass through the pore; this leads to insufficient analytical resolution. Herein, we present a new approach that slows down single-stranded DNA (ssDNA) and enables the detection of multiple separate bases. We show that chemical tags attached to bases cause a steric blockade each time a modified base passes through a narrow pore. The resulting characteristic current signatures are specific for the chemical composition and the size of the tags. Our approach for detection with modified DNA is independent of pore engineering and can potentially be applied to a wide range of solid-state nanopores to extend their sensing repertoire.^[2,3,14] This is a novel strategy because the detection is facilitated by the chemical modification of the analyte molecules rather than the engineering of pores.

This new approach for the base-specific identification of DNA was tested with the nonengineered version of the protein pore α -hemolysin (α HL; Figure 1 A). The α HL pore has been widely used in the past for the sensing of unmodified RNA and DNA strands.^[1,15] It therefore constitutes a good reference point for sensing with chemically modified DNA. α HL is of defined architecture with a lumen diameter of 1.3 nm at the narrow inner constriction and of 2 nm in the β barrel at the trans side of the pore (Figure 1 A).^[16] ssDNA

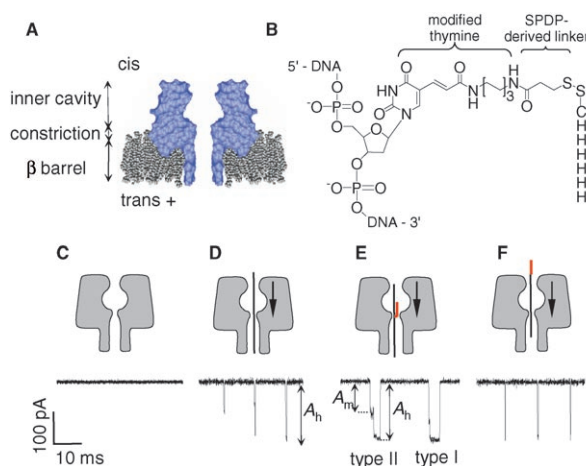


Figure 1. A) The α -hemolysin (α HL) pore embedded in a lipid bilayer; “+”: positive potential. B) The chemical linkage between DNA and the peptide within construct H_6C_1 -O1. C) Schematic representation of the α HL pore and a representative single-channel current trace. D) Events caused by oligonucleotide O1 without a peptide tag. E) Trace for the translocation of H_6C_1 -O1. F) Events for H_6C_1 -O1-term. (carrying the H_6C_1 tag at a terminal rather than an internal position). The traces were obtained from recordings in 2 M KCl and 20 mM tris(hydroxymethyl)aminomethane (Tris, pH 8.0), filtered and sampled at 10 and 50 kHz, respectively.

with an average cross-sectional diameter of 0.9–1.2 nm^[17] is known to pass the inner constriction.^[1,18] We speculated that chemical tags attached to separate bases would increase the cross-sectional diameter of the DNA and hence slow down translocation each time a modified base passes the narrow pore constriction. To test the approach, a model system consisting of commercially available DNA oligonucleotides and tags composed of peptides was used. Peptides were selected because their size, length, charge, and hydrophobicity can be easily tuned in a modular fashion to optimize pore blockades.

We first confirmed that a single peptide tag is capable of retarding strand translocation. DNA-strand oligonucleotide O1, of 27 bases in length, was modified with the hexahistidine tag H_6C_1 at an internal base (Figure 1 B; see the Supporting Information). The resulting peptide–DNA conjugate, H_6C_1 -O1, was analyzed in nanopore recordings. In the absence of DNA, the wild-type α HL pore yielded a conductance of (1900 ± 120) pS (number of independent recordings $n = 4$) when a positive potential was applied at the trans side (Figure 1 C and A). The addition of unmodified oligonucleotide O1 to the cis side of the pore (Figure 1 A) led to short high-amplitude events (Figure 1 D). These were characterized by an average amplitude of $(91.7 \pm 1.1)\%$ relative to the

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Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/ange.200800183>. It contains details of the synthesis of peptide-modified DNA strands, details of nanopore recordings, representative current events, additional data on the event analysis, and a discussion on the combined use of chemically modified DNA and solid-state nanopores.

open-pore current and had a duration, τ_{off} of (0.18 ± 0.06) ms ($n=3$). The short events represent the fast translocation of individual strands from the cis to the trans side of the pore (Figure 1D).^[1,18] The recordings also indicated blockages with 50 % amplitude, which were not pursued further as they likely represent the reversible threading of a strand into and the escape from the cis opening rather than complete translocation to the trans side.

When modified DNA strand $\text{H}_6\text{C}_1\text{-O1}$ was analyzed, events of two different types were observed. Type I events (Figure 1E) had a high amplitude, A_{h} , of $(96.8 \pm 0.5)\%$ with an average duration, $\tau_{\text{off-h}}$, of (1.83 ± 0.26) ms. Due to the nature of this very defined blockage, type I events certainly represent complete pore translocation. By comparison, type II events (Figure 1E) started with a medium amplitude, A_{m} , of $(56.6 \pm 2.6)\%$ with a duration, $\tau_{\text{off-m}}$, of (1.34 ± 0.36) ms. This medium level was followed (Figure 1E) by a high-amplitude blockage of $(97.4 \pm 0.9)\%$ with a duration of (1.96 ± 0.48) ms. The medium-amplitude blockage of type II events possibly stems from misfolded strands that reside in the internal cavity but eventually thread into the inner constriction. Due to the uncertainty in the assignment of the medium-level blockage, we focused our further investigations on the more clearly defined type I events, which only exhibited high-amplitude blockages. A comparison of type I events from $\text{H}_6\text{C}_1\text{-O1}$ with the results obtained with unmodified DNA shows that the histidine tag slowed down translocation by a factor of 10 and increased the current amplitude by 5 %.

Several lines of evidence support the notion that the current blockages with the histidine-modified strand are caused by the steric hindrance encountered when a wide peptide–DNA segment passes the narrow inner constriction

(Figure 1E). First, histidine tags with six, four, or two residues led to correspondingly shorter high-amplitude blockages in type I events (Table 1, $\text{H}_x\text{C}_1\text{-O1}$, $x=6, 4$, or 2 ; for type II events, see the Supporting Information). This implies that the peptide is elongated and aligned parallel to the DNA strand while being translocated. Second, tags composed of less-bulky glycine residues did not exhibit the same length dependence, which indicates that the smaller amino acid does not reach the critical size threshold required for continually slowing down DNA (type I events: Table 1, $\text{G}_x\text{C}_1\text{-O1}$, $x=6, 4$, or 2 ; for type II events, see the Supporting Information). Third, oligonucleotide O1 carrying an H_6C_1 tag at a terminal rather than an internal position did not greatly retard the DNA passage, as shown by the short event time of (0.23 ± 0.10) ms (Figure 1F; Table 1, $\text{H}_6\text{C}_1\text{-O1-term.}$). The absence of major retardation is attributed to the fact that the peptide can sequentially pass through the pore after the DNA strand, without the formation of a bulky peptide–DNA segment.^[19] The peptide tags are certainly the molecular reason for the retardation and may exert their effect by either hindered diffusion or an increase in friction^[20,21] mediated by steric, electrostatic, polar, and/or hydrophobic interactions.

Additional peptides were examined to demonstrate that strand retardation is a general feature of bulky amino acids and not only restricted to histidine residues. An additional aim was to identify tags that give rise to current signatures that are distinguishable from the histidine blockages. Two different peptides were investigated. The first peptide, R_7C_1 , was composed of seven arginine residues. In the nanopore analysis, type I events of $\text{R}_7\text{C}_1\text{-O1}$ (Figure 2A) exhibited a

Table 1: Characteristics of type I translocation events of DNA carrying a single chemical tag.^[a]

Modified DNA	A_{h} [%] ^[b]	$\tau_{\text{off-h}}$ [ms] ^[c]
$\text{O1}^{[d]}$	91.7 ± 1.1	0.18 ± 0.06
$\text{H}_6\text{C}_1\text{-O1}$	96.8 ± 0.5	1.83 ± 0.26
$\text{H}_4\text{C}_1\text{-O1}$	96.0 ± 0.6	1.57 ± 0.29
$\text{H}_2\text{C}_1\text{-O1}$	93.0 ± 0.7	0.82 ± 0.16
$\text{G}_6\text{C}_1\text{-O1}$	91.4 ± 0.6	0.56 ± 0.15
$\text{G}_4\text{C}_1\text{-O1}$	92.5 ± 0.5	0.55 ± 0.12
$\text{G}_2\text{C}_1\text{-O1}$	92.7 ± 0.4	0.53 ± 0.13
$\text{H}_6\text{C}-\text{O1-term.}^{[d]}$	93.9 ± 0.7	0.23 ± 0.10
$\text{H}_6\text{C}_1\text{-O1/pH 6.4}$	96.9 ± 0.7	2.18 ± 0.42
$\text{R}_7\text{C}_1\text{-O1}$	98.9 ± 0.6	25 ± 5
$\text{Y}_3\text{C}_1\text{-O1/step}$	$92.4 \pm 0.6/97.8 \pm 0.5$	$0.46 \pm 0.15/0.35 \pm 0.13$
$\text{Y}_3\text{C}_1\text{-O1/slope}$	94.8 ± 0.7	1.00 ± 0.22
$\text{Y}_3\text{C}_1\text{-O2/step}$	$91.8 \pm 0.6/98.9 \pm 0.7$	$0.43 \pm 0.12/0.39 \pm 0.08$
$\text{Y}_3\text{C}_1\text{-O2/slope}$	96.7 ± 0.8	0.97 ± 0.13

[a] The recordings were conducted in 2 M KCl and 20 mM Tris (pH 8.0), filtered at 10 kHz, and sampled at 50 kHz unless stated otherwise. The number of events analyzed for each type of DNA was between 1500 and 2000. $n=3$. [b] The relative amplitude was calculated by using $A = (I_{\text{OC}} - I_{\text{BC}})/I_{\text{OC}}$, in which I_{OC} and I_{BC} are the conductance levels from the open and blocked channel, respectively. I_{OC} and I_{BC} were derived by using all-point histograms. [c] The average duration represents the mono-exponential fit of the dwell-time histogram. [d] Filtered at 30 kHz and sampled at 100 kHz.

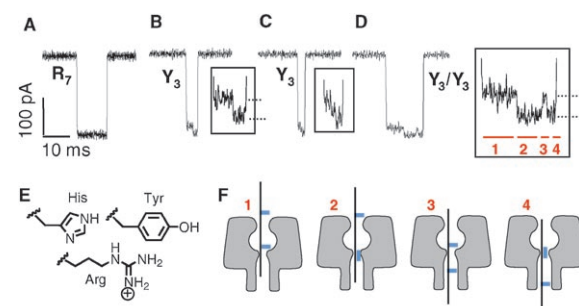


Figure 2. Representative nanopore translocation events for A) $\text{R}_7\text{C}_1\text{-O1}$, B) $\text{Y}_3\text{C}_1\text{-O1}$ exhibiting a current step, C) $\text{Y}_3\text{C}_1\text{-O1}$ exhibiting a current slope, and D) $\text{Y}_3/\text{Y}_3\text{-O3}$. The insets display magnified views of the high-amplitude regions of the events. E) Amino acid side chains of histidine, arginine, and tyrosine. F) Schematic representation to account for the current signature of the $\text{Y}_3/\text{Y}_3\text{-O3}$ events in (D).

duration of (25 ± 5) ms and an amplitude of $(98.9 \pm 0.6)\%$ (Table 1, $\text{R}_7\text{C}_1\text{-O1}$). This blockage has higher amplitude and is longer than that of $\text{H}_6\text{C}_1\text{-O1}$ (Table 1). The more pronounced blockade of $\text{R}_7\text{C}_1\text{-O1}$ is possibly due to the longer amino acid side chain of arginine compared to that of histidine (Figure 2E) or to the folding back of the positively charged arginine onto the negatively charged DNA backbone to generate a compact and bulkier DNA–peptide segment. The second peptide investigated was Y_3C_1 . Tyrosine has an uncharged aromatic side chain (Figure 2E). Translocation of

Y_3C_1-O1 led to type I events with two current levels (Figure 2B, inset, dotted lines). The first level at $(92.4 \pm 0.6)\%$ (Figure 2B, inset, top dotted line) is similar to the blockage amplitude of unmodified DNA. It is therefore very likely that this level stems from a DNA strand that is threaded into the inner constriction but is kept from passing through the β barrel because the bulky peptide has not yet entered the narrow pore region. The second level at $(97.8 \pm 0.5)\%$ (Figure 2B, inset, bottom dotted line) is ascribed to a state in which the peptide–DNA segment has entered the inner constriction and translocates the β barrel. The step signature was observed for 60% of type I Y_3C_1-O1 events. In the remaining events, the transition between the two current levels resembled a slope (Figure 2C, inset). This could reflect a peptide–DNA segment that is being gradually, rather than abruptly, pulled into the inner constriction. Importantly, the step-like blocking effect of Y_3C_1 was independent of the DNA sequence around the modified base because the same event characteristics were also seen for Y_3C_1-O2 with a different oligonucleotide sequence (Table 1; Y_3C_1-O1 /step versus Y_3C_1-O2 /step).

DNA strands with two separate chemical tags were tested to prove that tags act independently and give rise to correspondingly distinct current modulations. The first strand was a 37-mer Y_3/Y_3-O3 in which 2 Y_3C_1 peptides are tethered to 2 modified bases separated by 13 nucleotides. Similar to the results with the single-modified Y_3C_1-O1 strand, double-modified DNA gave rise to unresolved slope events (see Supporting Information) as well as fully resolved step-like events (Figure 2D). In the latter events, the blockage amplitude fluctuates twice between 2 levels, sequentially from event segments 1–4 (Figure 2D, event segments numbered in red). The average current levels for segments 1 and 3 and for segments 2 and 4 are approximately 92 and 99%, respectively (Table 2). The step-like signature is in line with

the peptides, showed similar current modulations (see the Supporting Information). The two current levels were identical within experimental error to the values observed for Y_3/Y_3-O3 (Table 2). The duration of event segment 3 was, however, longer for Y_3/Y_3-O4 than for Y_3/Y_3-O3 (Table 2). This positive correlation between duration and tag separation indicates that a longer DNA strand takes more time to pass the pore. The 37-mer Y_3/Y_3-O5 , with a separation of 7 nucleotides between the peptides, also displayed the step behavior. The percentage of stepped events, as well as the quality of the current steps, was lower for Y_3/Y_3-O5 than for Y_3/Y_3-O3 and Y_3/Y_3-O4 (see the Supporting Information). This reduced resolution agrees with molecular models showing that the tag with an extended length of 2.8 nm bridges the gap between the two tagged bases separated by 7 nucleotides or 2.2 nm.

In this study, we have developed a new nanopore-based strategy to enable the detection of separate bases in DNA strands. By using a model system of DNA oligonucleotides modified with peptides, we have demonstrated that chemical tags attached to bases are capable of causing characteristic current signatures for strands translocating through nanopores. The proof-of-principle experiments show that the blockage duration, amplitude, and signature can be tuned by changing the length, charge, and size of the tags. The current modulations are independent of the surrounding DNA sequence and two tags on a strand retain their characteristic signatures, which opens up the possibility of attaching multiple tags to DNA. To the best of our knowledge, this is the first time 1) that pore recordings have detected one or two separate bases in translocating individual DNA strands and 2) that chemically modified DNA has been used to infer base-specific information.

While our experiments have been performed with synthetic oligonucleotides, the approach can potentially be applied to sense DNA from biological samples. For example, peptide tags can be incorporated into copied DNA strands by using chemically modified nucleotides and sequence-specific primer extension. This approach would be suitable to sense the presence or absence of single-nucleotide polymorphisms by incorporating and detecting a modified base only if the target mutation is present. With further improvements in the tags, such as, a decrease in the size of the linker and the length of the tags, it will also be possible to reduce the nucleotide distance between the tags and thereby detect multiple bases in biologically relevant DNA strands. For example, the highly repetitive DNA regions in trinucleotide-expansion-disease genes could be sized by labeling the same base in all of the repeats.^[22] The extension of the technology towards sequencing by measuring the ionic-current modulation for each base^[1] would be very difficult to achieve due to the small distance between neighboring bases. This does not, however, limit the potential of this method, because the concept of slowing down DNA through chemical tags is new and can be applied to various related nanopore approaches. These include fluorescence- or ionic-current-based sequencing of DNA-derived designed polymers in which the spacing between individual bases has been increased^[23] (see the Supporting Information) or strategies that detect tunneling current.^[24]

Table 2: Characteristics of type I translocation events of Y_3/Y_3-O3 and Y_3/Y_3-O4 carrying tags separated by 13 and 27 nucleotides, respectively.^[a]

Segments	1	2	3	4
$O3 A_h$ [%] ^[b]	92.2 ± 1.1	99.7 ± 0.7	92.3 ± 0.9	99.7 ± 0.6
$O3 \tau_{off-h}$ [ms] ^[b]	3.43 ± 0.67	0.80 ± 0.22	0.26 ± 0.08	0.61 ± 0.15
$O4 A_h$ [%] ^[b]	92.8 ± 1.1	98.2 ± 0.8	91.1 ± 1.5	98 ± 0.7
$O4 \tau_{off-h}$ [ms] ^[b]	1.34 ± 0.75	0.64 ± 0.26	0.67 ± 0.21	0.74 ± 0.37

[a] The recordings were conducted in 2 M KCl and 20 mM Tris (pH 8.0), filtered at 10 kHz, and sampled at 50 kHz. [b] Defined as in Table 1.

the expectations for two Y_3C_1 peptides because one peptide is known to cause a blockage step from approximately 92% up to 98% (Table 1, Y_3C_1-O1 /step). The signature of Y_3/Y_3-O3 in Figure 2D strongly suggests that the current alterations reflect the sequential pulling of a DNA strand through the pore, as illustrated schematically in Figure 2F (red numbers correspond to the segments in Figure 2D).

The interpretation of the stepped events as sequential pulling is supported by the finding that the 54-mer DNA strand Y_3/Y_3-O4 , with a separation of 27 nucleotides between

The general approach of using chemically modified bases is especially relevant for DNA sensing with solid-state nanopores. These pores exhibit very high mechanic stability, which makes them ideally suited for rugged electrical sensor devices. Despite progress in their fabrication, solid-state nanopores cannot be engineered to the same atomic precision as the protein pore α HL. For example, inorganic pores are usually not narrow enough to discriminate between single- and double-stranded DNA; this thereby places constraints on their ability to detect DNA through a hybridization approach.^[9] The use of chemically modified DNA strands can address this limitation by tuning the cross-sectional diameter of ssDNA to existing pore dimensions rather than matching the pore dimensions to the size of the DNA strand. A more detailed discussion on the combined use of chemically modified DNA and solid-state nanopores can be found in the Supporting Information.

In summary, our strategy represents a new approach because it uses the chemical modification of the analyte, rather than pore engineering, to expand and enhance the sensing repertoire of nanopores. The concept can be applied to other bioanalytes.

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