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Chemically Labeled Nucleotides and Oligonucleotides Encode DNA for Sensing with Nanopores

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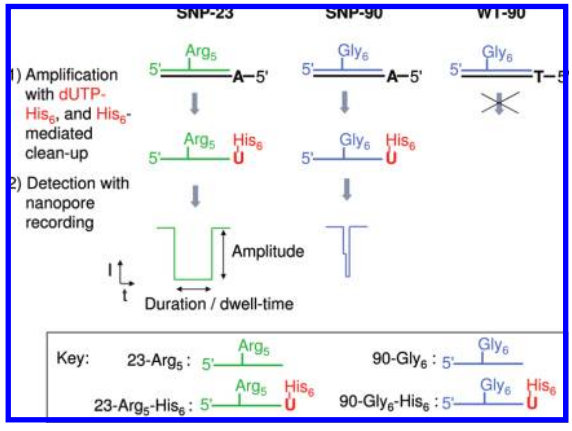
The labeling of nucleotides and oligonucleotides with reporter groups is an important tool in the sequence-specific sensing of DNA. Prominent examples include fluorescence tags that encode DNA sequences[1] or bases.[2] Reporter groups have also been used for the electronic detection of nucleic acids via cyclic voltammetry,[3] but not for the eletrical nanopore recordings. In nanopore recordings, individual DNA strands are electrophoretically driven through a nanoscale pore leading to detectable blockades of ionic current.[4] Existing nanopore approaches for the sequence-specific sensing of DNA rely on the formation of a duplex which cannot penetrate a narrow pore,[5−7] or the use of pore-linked receptors for single stranded DNA,[8,9] or individual nucleotides.[10] Here we present a new strategy which exploits tagged nucleotides[11] and oligonucleotides[12] for the sequence-specific sensing of single-point mutations. The chemical tags cause characteristic electrical signatures and thereby encode DNA sequences. By being independent of pore engineering, our approach might enhance the sensing repertoire of durable solid-state nanopores.[13]

The sensing approach was tested using the biologically relevant model system of single nucleotide polymorphisms (SNPs) in codons 23 and 90 of the HIV protease gene. The SNPs confer resistance against the HIV drug Nelfinavir.[14] The principle of the two-step sensing strategy is outlined in Scheme 1. First, the presence of the

Scheme 1. Sensing of SNPs Using Linear Amplification of Chemically Tagged Probe DNA Strands in Combination with Single-Molecule Nanopore Recordings



SNPs is encoded by chemically tagging DNA strands. Probe strands 23-Arg_5 and 90-Gly_6 bind sequence-specifically to the SNP-containing templates SNP-23 and SNP-90 (Scheme 1, step 1, templates in black) to facilitate the enzymatic addition of the hexahistidine-tagged nucleotide analogue dUTP-His_6 (red). This uridine nucleotide analogue is complementary to the resistance-conferring base A and is therefore only added for SNP-containing templates SNP-23 and SNP-90 (Scheme 1) but not wild-type WT-23 which carries the mismatched base C (Supporting Information, S6−S7) or base T for WT-90 (Scheme 1). By encoding for the presence of the SNPs, the His_6-tag facilitates the selective isolation of the SNP-derived DNA probes 23-Arg_5-His_6 and 90-Gly_6-His_6 using metal affinity purification (Scheme 1, step 1). In the second step, tagged DNAs are electrically detected in nanopore recordings (Scheme 1, step 2). Signals stemming from the two probe strands are discriminated because the covalently linked Arg_5 and Gly_6 tags give rise to two current signatures with different amplitudes and different average durations or dwell-times (Scheme 1).

The template-directed enzymatic extension was conducted with probe strands 90-Gly_6 and 23-Arg_5 (see Supporting Information, S3−S5) and dUTP-His_6 (Figure 1A). The His_6 tag of dUTP-His_6

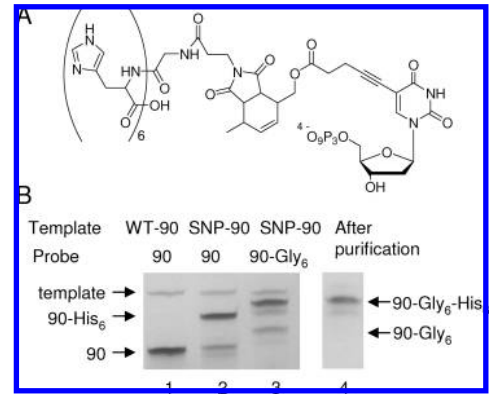


Figure 1. (A) dUTP-His_6; (B) gel electrophoretic analysis of the extension reactions. 90-Gly_6 contains a faster migrating impurity originating from the commercial DNA oligo preparation.

is positioned at C-5 of the pyrimidine base (Figure 1A) to avoid any interference with DNA polymerization.[11] The addition of the tagged nucleotide was confirmed by a visible shift in the electrophoretic migration of the bands for 90-Gly_6 and untagged control 90 (Figure 1B, lanes 3 and 2). Addition of the tagged base was specific for the presence of base A in the templates because no extension occurred for base T in codon position 90 (Figure 1B, lane 1). His_6-tag purification in spin-column format resulted in the isolation of 90-Gly_6-His_6 (Figure 1B, lane 4). Similarly, 23-Arg_5 was specifically extended for template SNP-23 and His_6-tag purified (Supporting Information, S7−S8).

23-Arg_5-His_6 and 90-Gly_6-His_6 were subjected to analysis via nanopore recordings to investigate the different current signatures. The recordings were conducted with the nonengineered version of the protein pore α-hemolysin (αHL) (Figure 2A) which is a standardized reference because of its atomically defined structure[15] and widespread use.[16,17] In the absence of DNA, the αHL pore gave rise to a unitary conductance of 1.976 ± 0.066 nS (n = 7)

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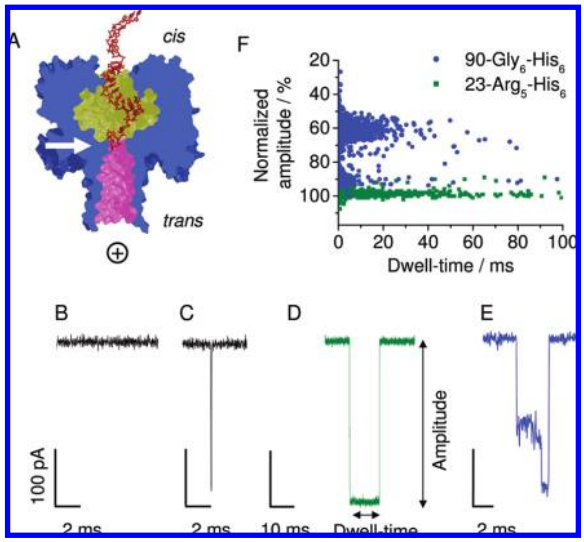

Figure 2. (A) The α-hemolysin (αHL) pore interacts with a ssDNA strand. The inner cavity and the β-barrel are highlighted in yellow and pink, respectively. The white arrow indicates the pore constriction. (B−E) Representative single-channel current traces for (B) αHL, and events of (C) untagged DNA 23, (D) tagged probes 23-Arg_5-His_6 and (E) 90-Gly_6-His_6. The traces were obtained from recordings in 2 M KCl and 20 mM TRIS HCl pH 8.0 at +150 mV, filtered, and sampled at 10 and 50 kHz, respectively. (F) Scatter plot for 23-Arg_5-His_6 and 90-Gly_6-His_6 events.

(Figure 2B). The addition of 23-Arg_5-His_6 to the cis side of the pore resulted in reversible current blockades (Figure 2D) which are most likely caused by the electrophoretically driven translocation of DNA strands to the positively polarized trans side of the pore (Figure 2A).[4,12] The blockades' characteristic average dwell-time, τ_{off}, of 9.33 ms and normalized amplitude, A, of 99% (Figure 2D, Table 1) is due to the Arg_5 tag as blockades of strands without a peptide tag were much shorter (τ_{off} = 0.16 ms) and of lower amplitude (91%) (Figure 2C). The signature of 23-Arg_5-His_6 events is in line with previous observations that a positively charged peptide folds back on the negatively charged DNA thereby creating a compact bulge which slowly translocates the narrow pore constriction.[12] The terminal His_6 tag, by contrast, sequentially passes after DNA and does not cause any slow-down.[12] In comparison, 90-Gly_6-His_6 gave rise to different current blockades which were characterized by a midlevel blockade followed by a high-amplitude blockade of 91% (Figure 2E)(Table 1). The midlevel originates from DNA strands which initially reside inside the wide inner cavity of the αHL pore but eventually thread into and translocate the narrow β-barrel of the pore (Figure 2A). The high-level blockades of 90-Gly_6-His_6 were shorter and of lesser amplitude than 23-Arg_5-His_6 because the Gly_6 is smaller than Arg_5 and causes less steric blockade.[12] The events of 90-Gly_6-His_6 clustered into well-separated groups within a scatter plot where each event is represented as a data point of defined amplitude and dwell-time (Figure 2F).

Table 1. Summary of Current Blockades for 23-Arg_5-His_6 and 90-Gly_6-His_6 in Nanopore Recordings

probe strand	τ_{off} (ms)[a]	A (%)[a,b]
23-R_5-H_6	9.33	99
90-G_6-H_6	0.76	91
	1.21 (13%)/8.07 (87%)[c]	59 (80%)/72 (20%)[c]

[a] Obtained as described in the Supporting Information (S8−S11). The values are the averages from three independent recordings each for 23-Arg_5-His_6 and 90-Gly_6-His_6, and a recording in which both modified DNA probes were present. [b] Amplitude, A, was normalized to the conductance of the open unblocked channel. [c] τ_{off} and A values for midlevel events of 90-Gly_6-His_6. Two values each are provided to reflect the two components in the respective distributions. The relative proportion of the components is given in brackets.

In summary, our new strategy to encode sequence information exploits our previous observation that chemical tags can slow down DNA translocation through a nanopore.[12] The strategy detects SNPs and is therefore not able to sequence de-novo. The electrical nanopore approach complements existing fluorescene-based SNP-sensing strategies,[18] and may be miniaturized for point-of-care applications. The sensing method is particularly relevant for solid-state nanopores.[13] These pores are more durable and rugged than protein pores which are inserted in more fragile lipid-bilayer membranes. The abiotic pores cannot, however, be engineered to atomic precision as the biological pores, thereby limiting their potential sensing repertoire. Our approach addresses this point as it senses without pore engineering. The strategy is currently able to discriminate between two sequences. To sense multiple DNA strands, tags can be further rationally designed[12] to achieve more characteristic current signatures. We note that multianalyte detection independent of pore engineering has previously been demonstrated for proteins.[19]

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Supporting Information Available: Complete ref 18; experimental details on the synthesis and chemical analysis of the tagged probe strands, primer extension of probe strands, and nanopore recordings. This material is available free of charge via the Internet at http://pubs.acs.org.

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