High-speed and High-resolution Readout of Multilevel Encoded DNA Origami by Sapphire-supported Nanopores

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Information-rich biomaterial has emerged as a novel platform for information storage, processing and secure communication. Solid-state nanopores have presented as an ideal label-free approach for barcoded double-stranded DNA (dsDNA) readout for large quantity of molecules. Nevertheless, compared to dsDNA, the encoded self-assembled DNA origami, which demonstrated multilayer encryption via scaffold sequence, length, message DNA strands hybridization and especially high-specific structural DNA folding, is a great candidate for high-security communication [1].

In this study, we demonstrated to use our lab-developed low-noise sapphire-supported (SaS) nanopore [2] to read out the message on the DNA origami with high accuracy and multilevel resolution. First of all, after hybridizing the message strands (M-strands) with the DNA scaffold, we decoded the information by stapling the scaffold into a tube-shaped four-helix bundle (4HB) (Figure 1a). Then the message was revealed by binding streptavidins (or DNA nanostructures) onto the message strands, which were finally read out by SaS nanopores in the ionic current measurement (Figure 1b).

We further advanced to design multilevel decoration on the DNA origami to promote the encoding capacity (Figure 2). 88% of the identified events demonstrated clear readout of all the five decorated spots. Under 250 kHz bandwidth high-speed (i.e. high-noise) reading, SaS nanopores still achieved a high signal-to-noise ratio (SNR) of 8.2 ($\Delta\overline{I_{2\times3WJ}}/I_{RMS}$), 10.1 ($\Delta\overline{I_{2\times4WJ}}/I_{RMS}$), 11.8 ($\Delta\overline{I_{4\times3WJ}}/I_{RMS}$) and 18.7 ($\Delta\overline{I_{2\times6WJ}}/I_{RMS}$) (3WJ: three-way DNA junction, 4WJ: four-way DNA junction, 6WJ: six-way DNA junction, Figure 3a). This enables successful resolving of the 2×3WJ, 2×4WJ, 4×3WJ, 2×6WJ according to the DNA nanostructure sizes statistically (Figure 3). By analyzing 114 current signals collected, we observed the normalized blockage current ($\Delta I_{WJ}/\Delta I_{4HB}$) and dwelling time ($\Delta t_{WJ}/\Delta t_{4HB}$) both follow the relation of 2×6WJ > 4×3WJ > 2×4WJ > 2×3WJ in their average (Figure 3b-c). This proves the feasibility of encoding and readout the DNA origami using the nanostructure sizes in addition to their location. Our demonstration paves way for high-capacity and high-security DNA cryptography with efficient and high-resolution readout.

References:

[1] Y. Zhang, *et al.* Nature communications **10.1**, 1-8 (2019).

[2] P. Xia, *et al*. Biosensors and Bioelectronics **174**, 112829 (2020).

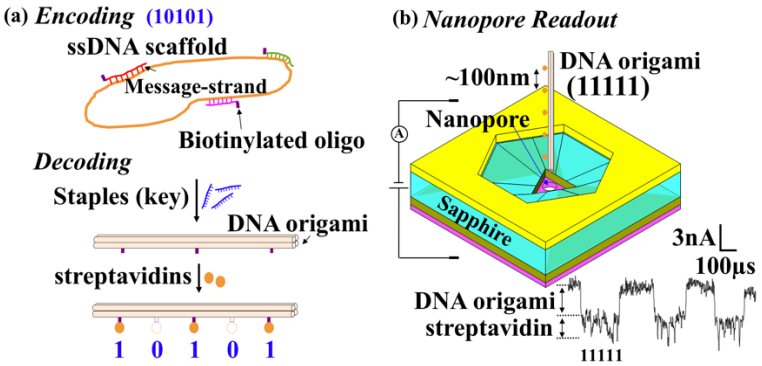

Figure 1. Encoding, decoding and sapphire-supported (SaS) nanopore readout of the messages in a DNA origami. (a) Encoding: A group of message strands (M-strand) with biotinylated oligo overhangs are hybridized onto a single-stranded DNA scaffold. Decoding: A set of staples as the key are used to fold the scaffold into the pre-defined DNA origami shape. Then the hidden message is revealed by binding streptavidins onto the biotinylated oligo overhangs. (b) The nanopore readout of the decoded messages (11111) by ionic current measurement.

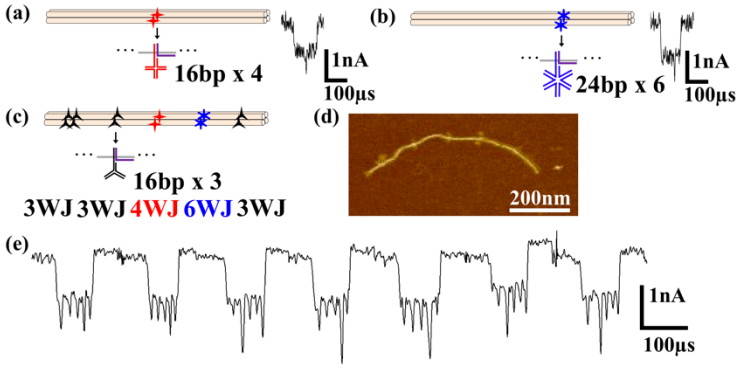

Figure 2. Multilevel DNA origami encoding and nanopore readout. (a-b) DNA origami encoded with a single 4WJ (four-way DNA junction) or 6WJ and a signal read out by SaS nanopores, respectively. (c-d) Multilevel DNA origami encoding scheme and a AFM image demonstrating the successful binding at all the five spots. (e) The first seven observed events during the readout of the DNA origami in figure c by a SaS nanopore.

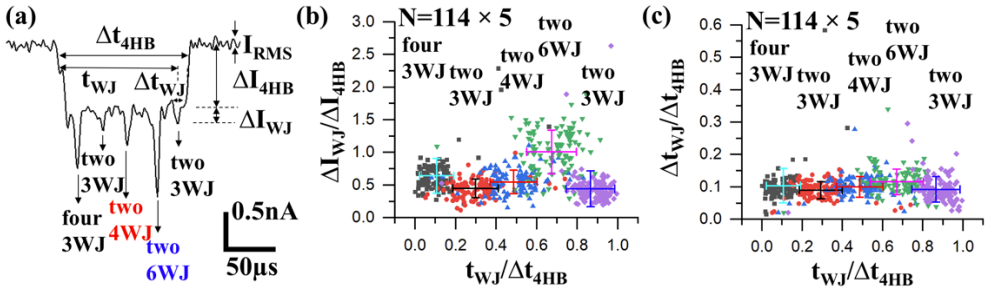

Figure 3. Resolving multilevel encoded DNA origami in nanopore reading. (a) A nanopore readout event demonstrating the different blockage current (ΔI_{WJ}) of the four 3WJ, two 3WJ, two 4WJ and two 6WJ. (b-c) Statistical analysis of 114× 5 readout events successfully resolving the WJs with different nanostructure sizes in both $\Delta I_{WJ}/\Delta I_{4HB}$ and $\Delta t_{WJ}/\Delta t_{4HB}$ (2×6WJ > 4×3WJ > 2×4WJ > 2×3WJ).