A single protein resolution mapping of DNA-ZFP interaction using solid-state nanopores

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Solid-state nanopore has been widely studied as an effective tool to detect and analyze small bio-molecules, such as DNA, RNA, and proteins in a single molecule level. Here, we demonstrated a rapid mapping of zinc finger protein (ZFP) which is bound to a specific locus along the length of a double-stranded DNA (dsDNA) to single protein resolution using a low noise solid-state nanopore. When ZFP labeled DNAs were driven through a nanopore by an externally applied electric field, characteristic ionic current signals arising from the passage of DNA/ZFP complex and bare DNA were detected, which enabled us to identify the locations of ZFP binding site. We examined two DNAs with ZFP binding sites at different position and found that the location of additional current drop derived from DNA/ZFP complex is well matched with theoretical one along the length of DNA molecule. The results suggest a possibility of mapping the location of protein binding site on DNA or reading off a lot of genetic information to a single molecule level using solid-state nanopores.

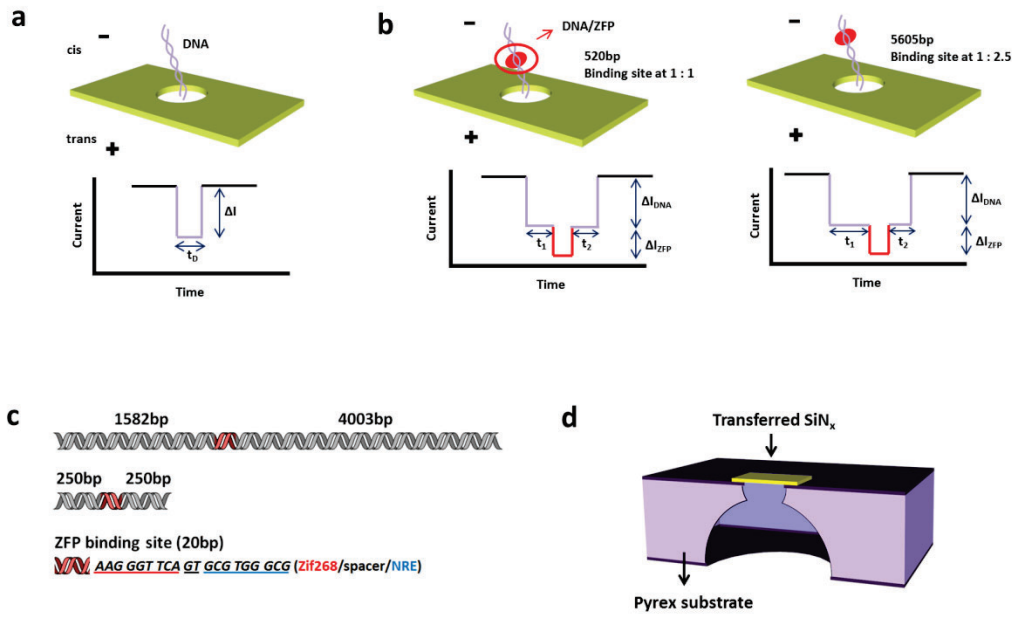

Figure 1. Schematic illustrations of nanopore based mapping of DNA-protein interactions. (a) A translocation event of a bare dsDNA gives rise to a single level current drop. (b) Two dsDNAs having ZFP binding sites at different part of the strands show an additional current drop peak in accordance with the binding site. (c) Schematics of two dsDNA used in this work. One is 5605 bp with 20 bp binding site at the position of 2-to-5 and the other is 520 bp with 20 bp binding site at the center. (d) A cross-sectional illustration of a low noise solid-state nanopore device used in this work (not to scale).

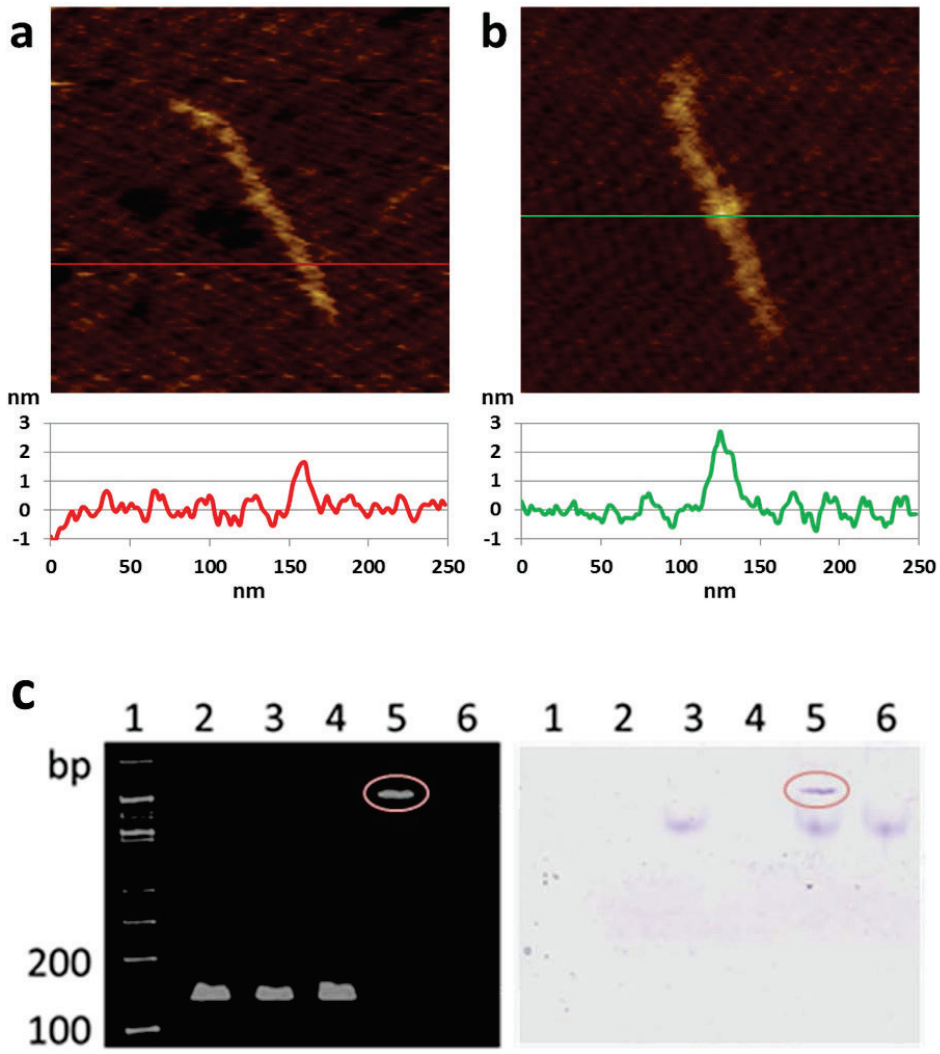

Figure 2. AFM images of bare DNA (a) and DNA-ZFP complex (b). DNA of 520 bp having about 180 nm length was used in this analysis. The scan sizes of the images are 250 × 250 nm with Z-scale of 4 nm. (c) Gel-shift assay of bare DNA and DNA/ZFP complex run on 10% native polyacrylamide gel and stained with EtBr (left) and coomassie blue (right). Lane 1: DNA marker. Lane 2: DNA (134 bp) without recognition sequence. Lane 3: DNA (134 bp) without recognition sequence reacted with ZFP. Lane 4: DNA (126 bp) with recognition sequence. Lane 5: DNA (126 bp) with recognition sequence reacted with ZFP. Lane 6: ZFP. The protein bands in lower position are from unbound free ZFPs.

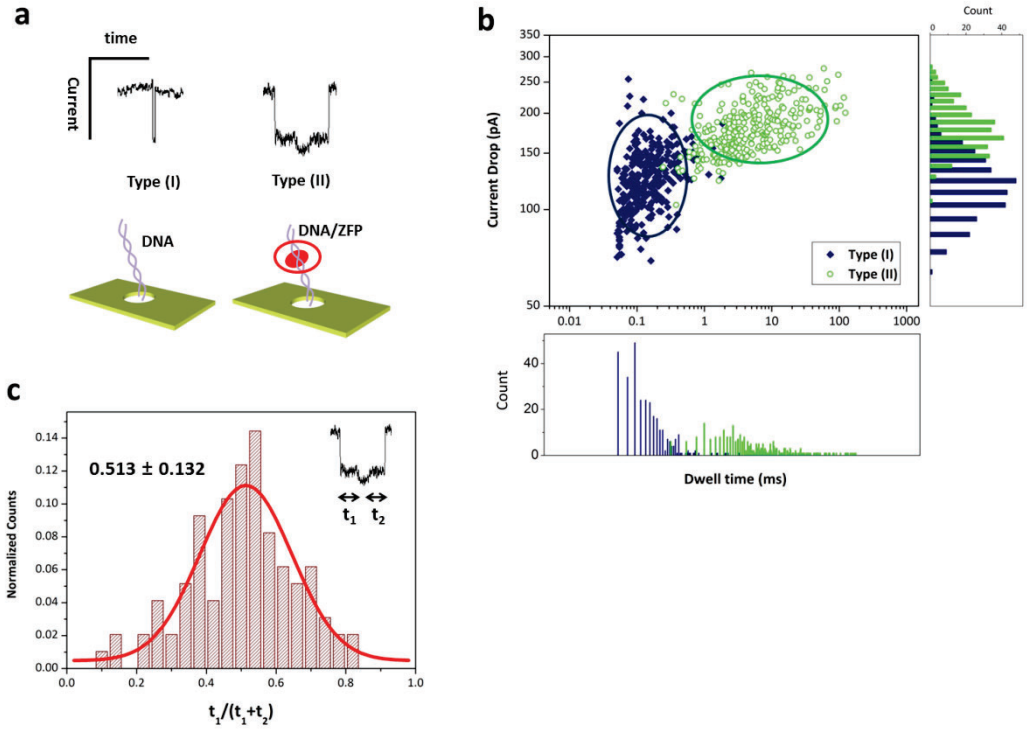

Figure 3. Analysis of translocation events for DNA (520 bp, binding site at the center) with ZFP. (a) Two types of event signals were detected. Type (I): signals with a single profile current drop. Type (II): signals with an additional current spike. (b) Scatter plot of translocation events. Type (I) and type (II) events were indicated as navy diamonds and green circles, respectively. Type (II) events clearly show higher current drop and longer dwell time. (c) Histogram of $t_1/(t_1+t_2)$, where t_1 is assigned as the dwell time from the start of event to the additional spike and t_2 is assigned as the dwell time from the additional spike to the end of event.

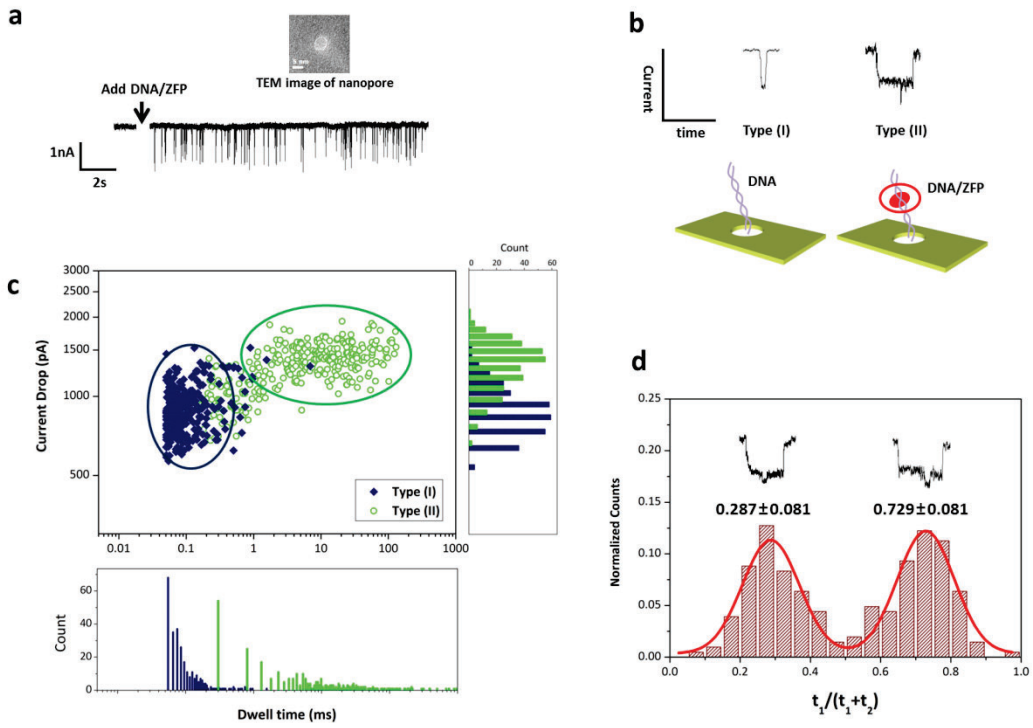

Figure 4. Analysis of translocation events for DNA (5605 bp, binding site at 2-to-5 position) with ZFP. (a) Current traces of DNA (5605 bp)/ZFP translocations in 500 mM KCl solution when 500mV bias is applied across the nanopore membrane. A TEM image of a 7 nm pore drilled in a silicon nitride membrane with 20 nm thickness. (b) Representative translocation signals from bare DNA (Type I) and DNA/ZFP (Type II). (c) Scatter plot of translocation events of DNA (5605 bp)/ZFP. Type (I) events were indicated as navy diamonds and type (II) events were indicated as green circles. (d) Histogram of $t_1/(t_1+t_2)$ where t_1 is assigned as the dwell time from the start of event to the additional spike and t_2 is assigned as the dwell time from the additional spike to the end of event. Two Gaussian distributions were observed due to two opposite directions of translocations.