Direct Force Measurements on Double-Stranded RNA in Solid-State Nanopores

Supporting Information

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Methods

Position detection

Here we describe the bead position detection and its translation into force measurement. Additional details regarding the experimental configuration can be found elsewhere [1-3]. Position detection of the bead in the optical tweezers is facilitated by monitoring the reflection signal of a separate detection laser incident on the bead. The reflected light is directed onto a quadrant photo detector (QPD), which following amplification converts the total incident light into a voltage signal. The resulting signal is related to the position *z* of the bead with respect to the nanopore membrane (where *z* is defined in Figure **S1a**). Therefore, for each bead that is captured in the optical trap a calibration curve is recorded: using a piezo-controlled stage, the bead is moved slowly away from the membrane, while the QPD signal is recorded. As the piezo position is recorded simultaneously with nanometer precision, a calibration curve between the piezo position and the QPD signal is established. An example of such a curve is given in Figure **S1b**. The detailed shape of this curve is unique for each bead, and is determined by interference of the detection laser light between the membrane surface and bead surface. Since a single value of the QPD signal can correspond to multiple positions in *z*, camera detection is additionally employed to establish the absolute distance between the bead and the nanopore membrane [2].

Immediately after a calibration curve is recorded, the bead is moved in close proximity to the nanopore membrane. Using the just recorded calibration curve and the position information from the piezo controller and camera detection this initial position can be determined very accurately. A molecule attached to the bead can then be captured into the nanopore by application of a bias voltage over the nanopore. Upon capture, the bead will move, and the QPD signal will change accordingly, following the same path as the calibration curve. After capture, the voltage is varied, while still recording the QPD signal. In Figure **S1c** the voltage is stepwise increased from 10 mV to 110 mV, thereby increasing the net forces on the molecule inside the pore, which causes movement of the bead towards the nanopore membrane. This movement is reflected by the change in the QPD signal, which changes from a starting value of approximately 2.25 V (at V_{bias} = 10 mV) to a final value of 1.7 V (at V_{bias} = 110 mV). As the initial

position of the bead prior to capture of the molecule in the nanopore is known, the subsequent bead positions at different bias voltages can be determined for each voltage by following the changing QPD signal along the calibration curve. This is indicated by the different symbols in Figures **S2b** and **S2c**. In this particular case, the bead is moved by a total distance Δz of approximately 130 nm when the bias voltage is changed from 10 mV to 110 mV. This distance can be converted to a force using the stiffness of the optical trap k_{trap} as determined from a Lorentzian fit to the power spectrum of the bead position, using the relation $F = k_{trap} \cdot \Delta z$.

Determination of the position of the nanopore membrane

The exact position of the nanopore membrane can only be determined by physically moving the bead against the nanopore membrane, which dramatically changes the fluctuations in the PSD signal. Unfortunately, this can lead to aspecific attachment of the bead to the surface and can permanently change the conduction and current noise of the nanopore. However, we have found that the PSD noise signal increases about an order of magnitude when the bead is within approximately 100 ± 30 nm (error being the observed variation in where this crossover occurs over many different nanopores) from the membrane surface. This clear change in the QPD signal can be used to estimate the distance to the membrane with a precision of approximately 30 nm.

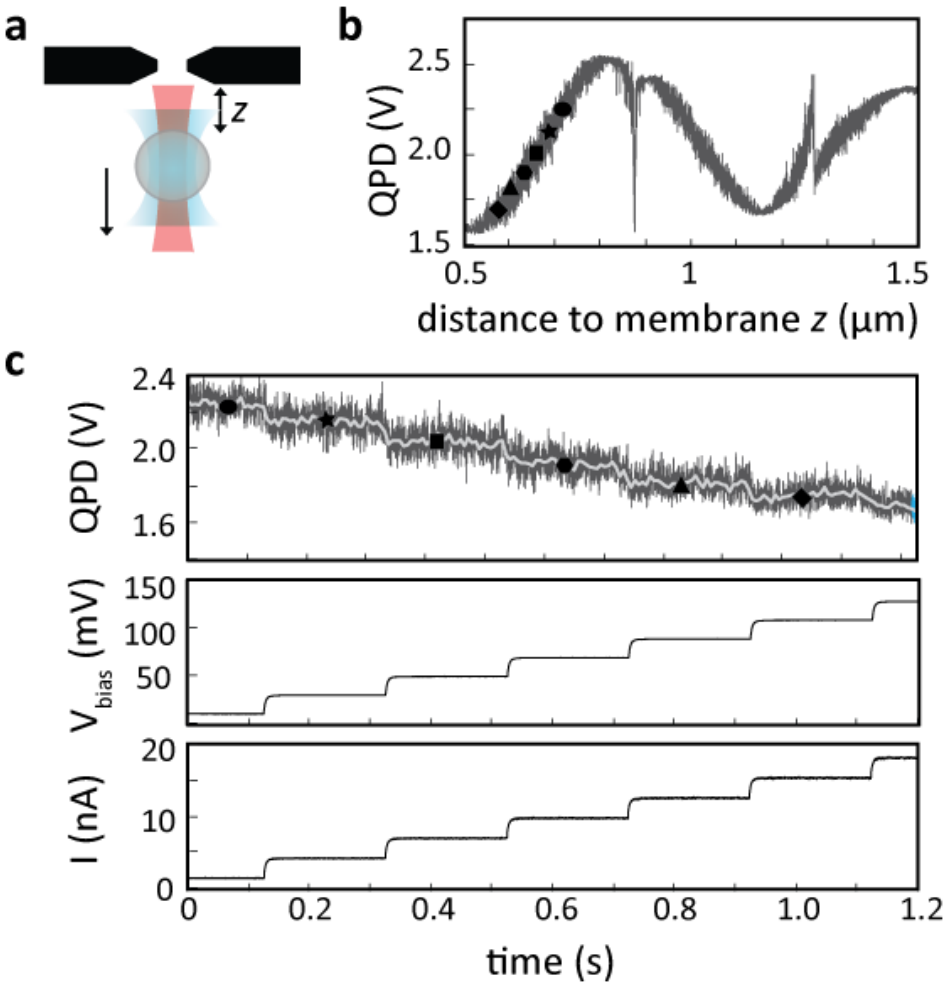

Figure S1: Position detection. a. Schematic defining the position *z* as the distance between the nanopore membrane surface and the bead surface. Prior to a measurement, the bead is moved away from the nanopore membrane surface while the position signal is recorded. **b.** Recorded QPD signal as a function of the position *z*. This curve is used as a calibration file relating QPD signal to position. **c.** After a molecule is captured in the pore, the voltage can be varied, leading to a commensurate change in bead position: the QPD signal (top) changes together with the bias voltage (middle), and the nanopore current (below). Using the calibration curve in **b** recorded previously, the QPD signal can be related to a position.

Signal-to-noise in determination of position

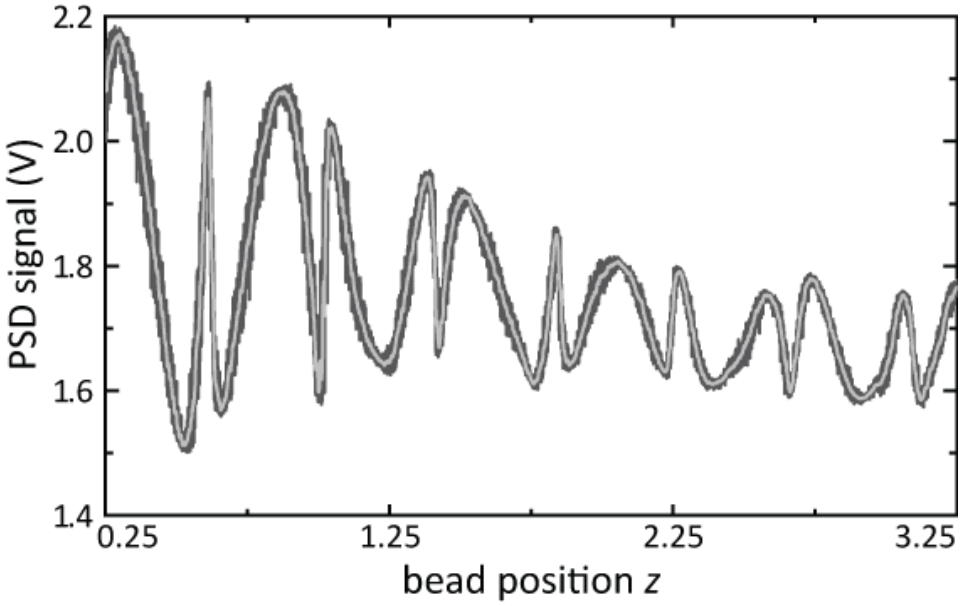

Figure S2: Calibration curve over a distance of 3 µm. The calibration curve shown here starts from a distance *z* of approximately 250 nm away from the nanopore membrane surface. Clearly, the sensitivity of QPD signal is higher when the bead is closer to the surface: at *z* = 0.25 µm, the QPD response is approximately fourfold larger than at *z* = 3.25 µm. This increases the signal-to-noise in determining the bead position at these small distances from the membrane, which is advantageous when working with short molecules.

References

1. Keyser, U.F., et al., *Direct force measurements on DNA in a solid-state nanopore.* Nature Physics, 2006. 2(7): p. 473-477.
2. Keyser, U.F., et al., *Optical tweezers for force measurements on DNA in nanopores.* Review of Scientific Instruments, 2006. 77(10).
3. Keyser, U.F., et al., *Inserting and Manipulating DNA in a Nanopore with Optical Tweezers.* Micro and Nano Technologies in Bioanalysis: Methods and Protocols, 2009: p. 95-112.