DNA Tunneling Detector Embedded in a Nanopore

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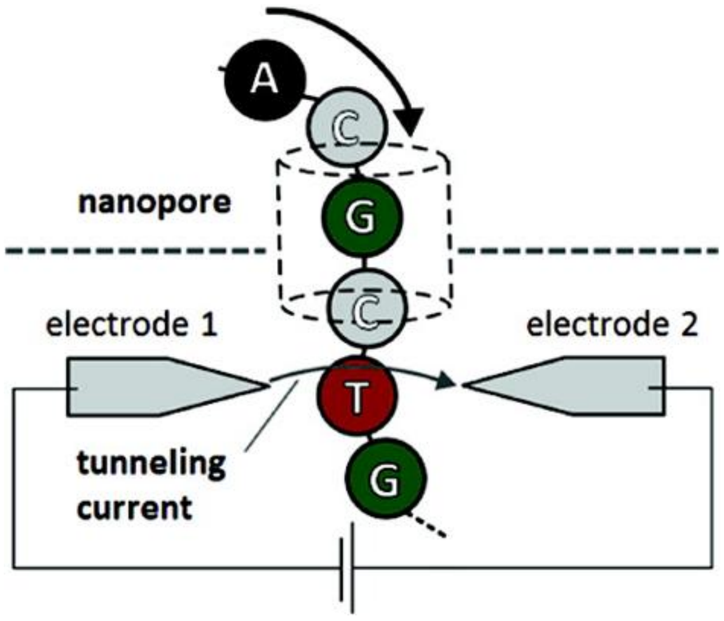
The accurate detection and analysis of single molecules in free-solution environments is one of the significant challenges for physical and biological sciences. In recent years, nanoscale pores have become a reliable platform for the discrimination of bio-polymers at the single molecule level. It has been suggested that if a nanopore can be combined with transverse tunnelling current detection, such technology can provide a fast, label-free, reliable and inexpensive method for DNA sequencing. Unfortunately, the exact alignment of nanoscale tunnelling electrodes to a nanopore has proven to be a significant challenge. We have developed a method based on electron-beam-induced deposition to fabricate such combined nanopore/tunneling junctions and have performed DNA translocation experiments using ds λ-DNA (48.5 kbp) as test system. Importantly, our setup allowed for concurrent detection of ion current blockades and tunneling current modulation as DNA passes through the junction. While further efforts are required to optimize device performance, in our view, this work represents an important step towards high-speed DNA sequencing by tunneling.

Why tunneling?

Tunneling is a quantum-mechanical effect, essentially based on the overlap of electronic wave functions: Electrons are able to "tunnel" across a gap between two electrodes, provided this gap is small enough (on the order of a few nanometers). The larger the electronic overlap, the higher the tunneling probability. Since the wave functions decay very rapidly with distance away from the metal surface, the tunneling effect is very distance-dependent, which is the reason for the high spatial resolution achieved in Scanning Tunneling Microscopy. Moreover, it depends on the electronic properties of the medium in the tunneling gap, thus introducing some degree of "molecular" specificity.

- **High spatial resolution**
- **Molecular specificity**



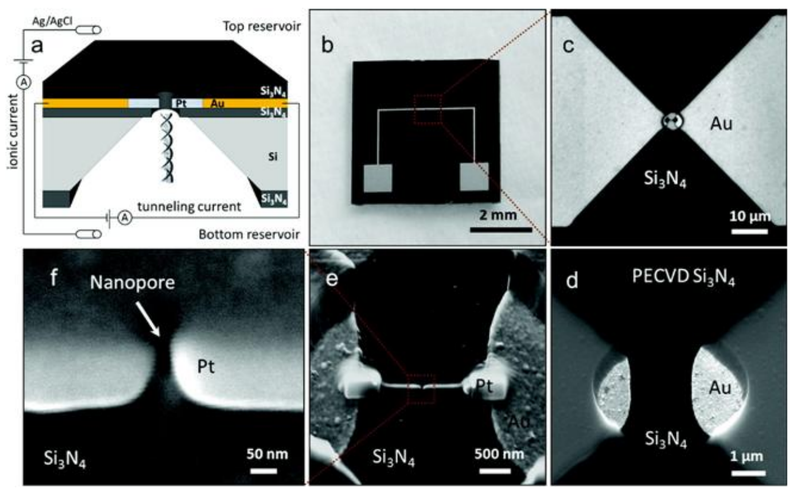
With a view on DNA sequencing, tunneling detection offers interesting prospects: If one could distinguish the different bases within a DNA strand, then one would "just" have to move the strand across the tunneling detector (or vice versa) and read off the base sequence. This implies that the polymeric chain needs to be in a linear configuration.

How do we achieve that?

One way of unfolding an individual DNA chain is to pull it through a sufficiently small aperture, a so-called "nanopore". Combined with a tunneling junction, such an architecture provides both: a means to force the DNA into a linear configuration and a probe to detect the tunneling current in real-time, i.e. as the DNA is pulled through the nanopore. However, given that the relevant size regime is in the low nanometer range, the fabrication of such a combined nanopore/tunneling junction is not trivial.

Device Fabrication and Characterization

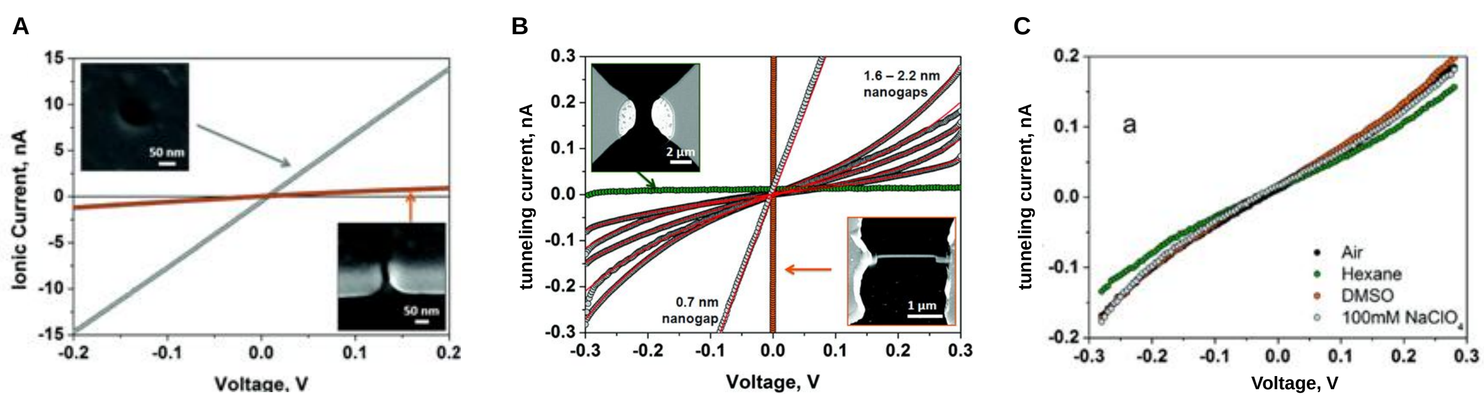
The figure below shows the device design at different levels: Panel a) Overall schematic; panels b)-f): Optical and electron microscopy images of the chip, inc. Au contact pads and nanopore/tunneling junction.



Pt electrodes are fabricated using electron-beam-induced deposition (EBID). The nanopore and the tunneling junction are characterized by electron microscopy, as well as ion conductance measurements and tunneling spectroscopy, respectively. Note that two Ag/AgCl electrodes are used to drive the DNA through the nanopore, where the two "on-chip" Pt electrodes form the tunneling junction.

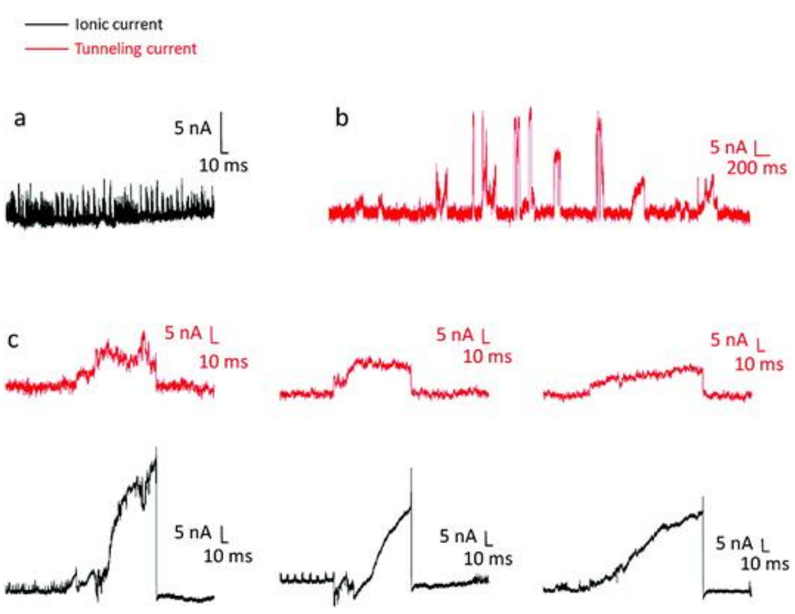
Panel A (below): Ion current/(Ag/AgCl electrode) bias traces before and after electrode deposition

Panels B and C: Tunneling current /bias curves for different devices (**B**) and in different media (**C**, typical example)



Concurrent ion current/tunneling current detection of translocating DNA

Upon addition of λ-DNA to the liquid cell, characteristic events are observed in the ion current (black) as well as the tunnelling current trace (red) (tunneling bias = 0.3 V, Ag/AgCl bias = -0.8V; ion current detection: Axopatch 200B, tunneling current detection: Gamry Ref600, 1M KCl + EDTA/HCl buffer).



Statistical analysis reveals two types of events:

Type I: (~ 97.5% (~3400) of all events for the currently used devices)
- Occur only in the ion current trace, tunneling current modulation is either too small, too fast or does not occur because DNA by-passes the tunneling junction during translocation.
- Mean dwell time 0.3 ± 0.2 ms corresponding to a translocation speed of 5.5 cm/s. This is in good agreement with previous work on Si_3N_4- and metallic nanopores (cf. Branton et al., 2-6 cm/s, Nano Lett. 2004, 4, 2293-2298 and Ayub et al., J. Phys.: Cond. Mat. 2010, 22, 454128). **DNA adsorption to the Pt electrodes not prominent** here.
- Mostly likely "normal" DNA translocation events

Type II: (~ 2.5% (~108) of all events for the currently used devices)
- Occur in both in the ion current (blockade) and the tunneling current (increase)
- Mean dwell time is on the order of 5 ms with a relatively large spread. On average, however, these events are about 20 times longer than the type I events.
- Apparently, DNA is interacting with the tunneling junction, but the exact nature of this interaction is not yet clear. It may include physical entanglement of the DNA strand with the tunneling electrode; adsorption of DNA to the metal surface; structural alteration of the DNA strand (transition from double- to single strand?)

Remaining issues and Outlook:
- Relatively high by-pass rate, which may be related to the device design
- Noise performance and acquisition speed
- Introducing a well-defined chemical interaction between the DNA bases and the tunneling junction

Further experiments are needed to fully understand the obervation made. However, with a success rate of roughly 10% for the device fabrication, we have established an approach to making combined nanopore/tunneling junctions on a routine basis. This is important prerequisite for implementing a nanopore-based DNA sequencing-by-tunneling device.

Related work by the Albrecht and Edel groups @ Imperial:

A. P. Ivanov et al., "DNA tunneling detector embedded in a nanopore", Nano Letters 2011, DOI: 10.1021/nl103873a (publ. on-line 06th Dec. 2010).
M. Ayub et al., "Precise electrochemical fabrication of sub-20 nm solid-state nanopores for single-molecule biosensing ", J. Phys.: Cond. Mat. 2010, 22, 454128. (Special Issue on Nanopore Sensing!)
M. Ayub et al., "Nanopore/electrode structures for single-molecule biosensing", Electrochim. Acta 2010, 55, 8237-8243.
G.A.T. Chansin et al., "Single Molecule Spectroscopy Using Nanoporous Membranes", Nano Letters 2007, 7, 2901–2906.

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