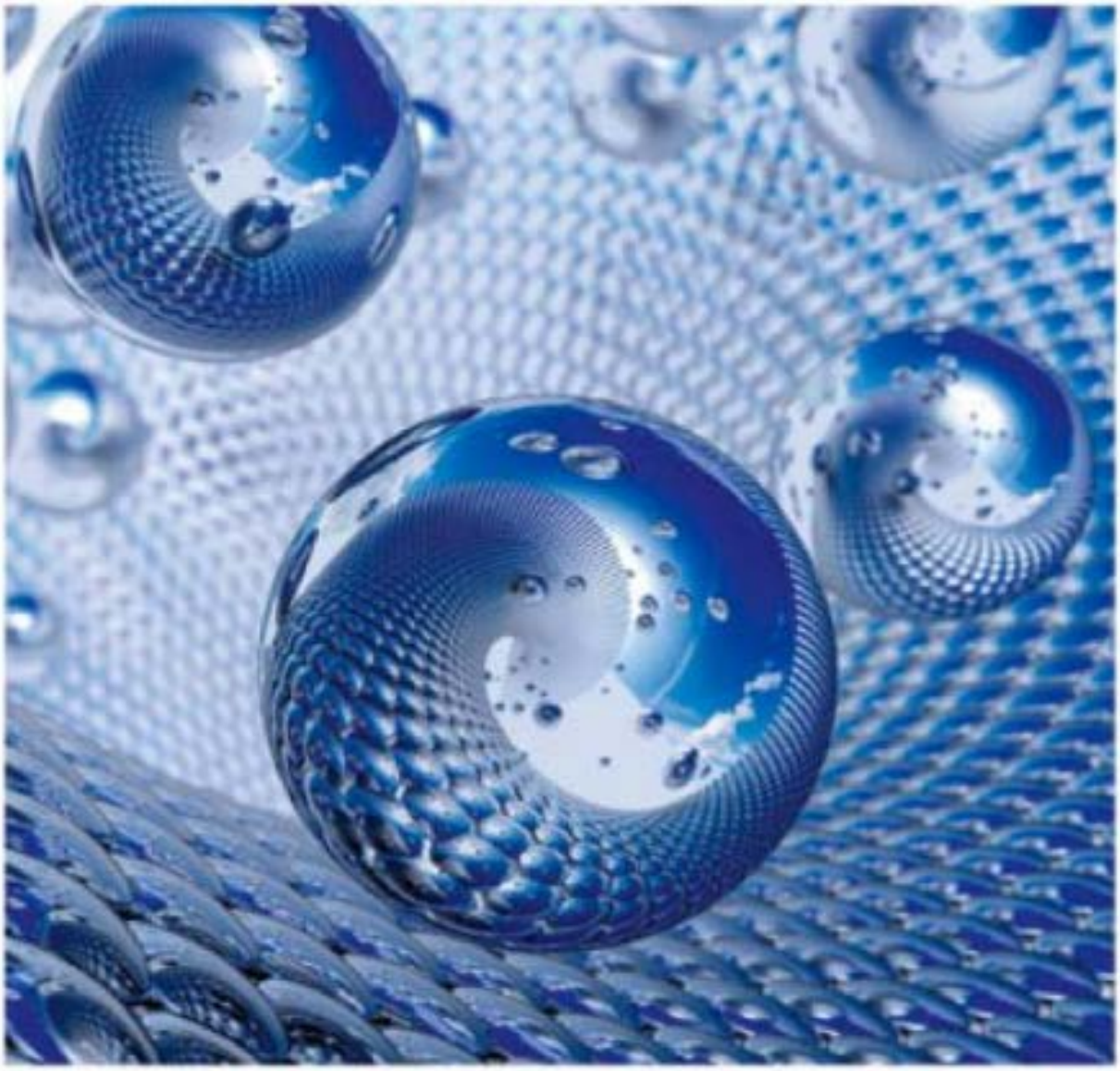
Edited by Joshua Edel and Tim Albrecht

Nanopores for Bioanalytical Applications

Proceedings of the International Conference



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SINGLE-MOLECULE DNA TRANSLOCATION THROUGH Si_3N_4- AND GRAPHENE SOLID-STATE NANOPORES

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1 INTRODUCTION

The controlled translocation of a single, double-stranded DNA (dsDNA) through a solid-state nanopore (NP)[1,2] with optical tweezers (OT) is described in the presence of an electric field under buffer conditions. Upon threading dsDNA complexed by single proteins through a NP in 20 nm thick Si_3N_4-membranes, we find distinct asymmetric and retarded force signals that critically depend on the overall charge of the protein, the molecular elasticity of the dsDNA and the counter-ionic shielding of the polyelectrolyte (dsDNA) in the buffer[3]. This force response can be quantitatively simulated and understood within a theoretical model where an isolated charge on an elastic, polyelectrolyte strand experiences a harmonic nanopore potential during translocation. In order to extend these experiments to atomically thin solid-state NP, dsDNA was threaded through single nanolayer graphene NP by a transmembrane voltage. Whereas distinct single-molecule threading signals could here be observed in a Coulter counter setup (Fig. 1a) and compare well with recent papers[4,5], OT controlled dsDNA-translocation through graphene NPs remained challenging, however (Fig. 1b). In this paper, we will give a current status report on optical tweezers controlled translocation through solid-state NPs.

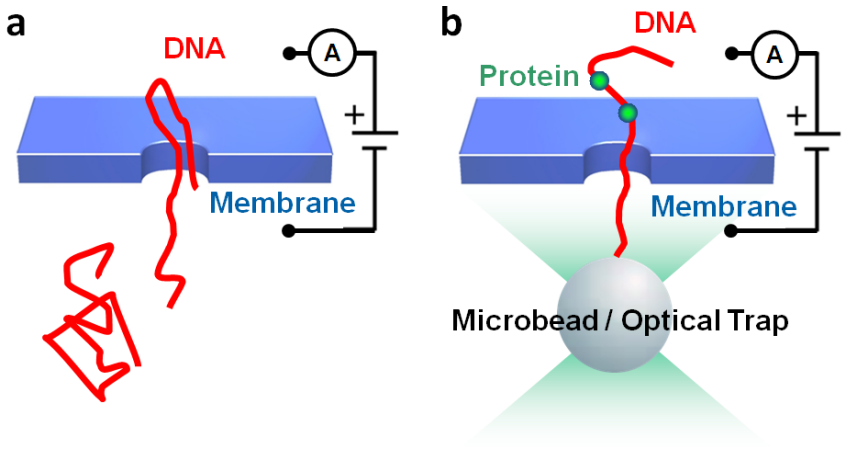

Figure 1 *(a) Coulter counter set-up for single-molecule NP translocation and ionic current detection. (b) Optical tweezers setup for measuring and controlling dsDNA NP translocation.*

2 METHOD AND RESULTS

2.1 Optical Tweezers Setup and Experimental Procedure

We used a single-beam, 3D quantitative OT system with confocal light guiding that incorporates an optical obstruction filter eliminating all axial light components and allowed manipulation and interference-free steering of polystyrene (PS) microbeads under buffer conditions in the vicinity of a reflecting interface (membrane)[6]. Individual Lambda (λ) bacteriophage dsDNA molecules (48502 basepairs (bp), 16.4 µm contour length) were functionalized at one end with several biotins and individually attached to a streptavidin-coated PS bead (3,28 µm diameter). The dsDNA-bead constructs were kept in buffer solution (20 mM / 1M KCl and 2 mM Tris/HCl at pH 8.0) at 22°C and introduced into our NP fluidic cell prior conducting the experiment. In order to probe the force response of an individual protein attached to dsDNA when being threaded through a NP, we introduced either EcoRI (31 kDa) or RecA (38 kDa) proteins.

2.2 Nanopore Fabrication

Solid-state NPs in 20 nm thick Si_3N_4- and single nanolayer graphene membranes were produced by helium-ion microscope milling (Orion, Zeiss, Oberkochen), rendering NP openings with diameters typically 20-60 nm in size.

2.3 Graphene Preparation and Electrical Characterization

Single and multiple graphene nanolayers have been mechanically exfoliated and transferred onto a 20 nm thick Si_3N_4-membrane via the "wedging transfer" technique[5,7] over a 5 µm wide hole that was etched into the Si_3N_4-membrane. Since special emphasis has been put on virtually void-free graphene nanolayers for high electrical transmembrane resistance, we used pristine, naturally grown single-crystal graphite nanoflakes (NGS Naturgraphit, Leinburg). In Fig. 2a, a graphene sample with one to three nanolayers is shown in a light microscopy image. A representative current-voltage (I-V) characteristics of a single nanolayer graphene is shown in Fig. 2b, exhibiting an electrical resistance of 29 GΩ in 20 mM KCl buffer solution (Gigaohm seal) as it could only be found in the measured pristine graphene flakes. A 20 nm wide, single nanolayer graphene NP is shown in Fig. 2c. The extraordinary image

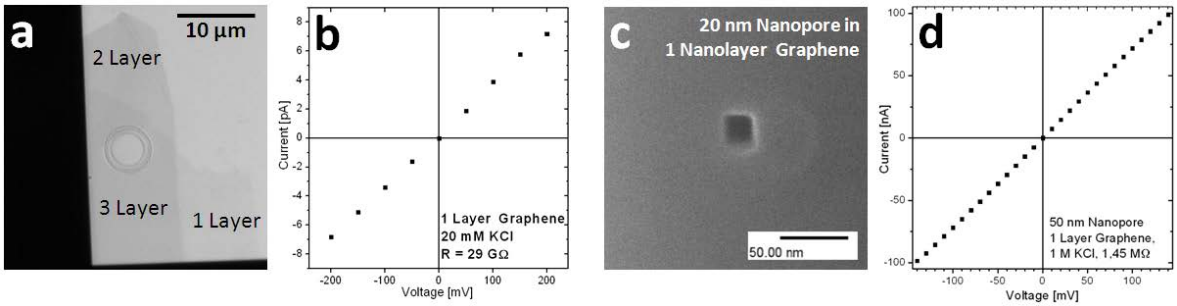

Figure 2 *(a) One to three nanolayer graphene sample transferred to Si_3N_4-membrane. (b) I-V curve of single nanolayer graphene with Gigaohm seal. (c) 20 nm wide NP fabricated in graphene nanolayer by He-ion microscopy milling. (d) 1,45 MΩ contact resistance of 50 nm single nanolayer graphene NP recorded in 1M KCl.*

contrast is due to the He-ion microscope technology that has also been used to drill the NP. In Fig. 2d, an I-V-curve of an 50 nm wide graphene NP is shown, exhibiting an NP resistance of 1,45 MΩ recorded in 1M KCl. In that respect two aspects are worth noting: 1) although control experiments always indicated proper NP fabrication, rather frequently we failed to measure a distinct NP electrical contact resistance even when occasionally surfactants were added to the solution to improve surface wetting, and 2) the measured NP resistance complies well with other data[5] extrapolated for a 50 nm NP.

3 RESULTS AND DISCUSSION

3.1 dsDNA Coulter Counter Experiments

After adding linear λ-DNA to the cis-compartment of our microfluidic cell device[6], and applying an electrical transmembrane voltage (trans: positive, cis: negative) reproducible single-molecule DNA translocation events could be discerned for both Si_3N_4- as well as single nanolayer graphene NPs. In Fig. 3a and b, representative translocation signals are given, reflecting the expected ionic-current blockade during dsDNA translocation for 1 M KCl buffer solution. As already indicated in previous publications, the current variations reflect the folding configuration of the translocating dsDNA-molecules[4,5,8].

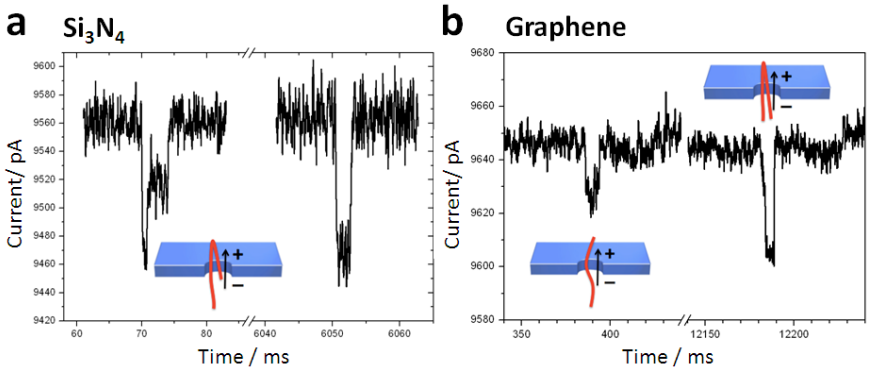

Figure 3 *(a) Coulter counter signals of two λ-DNA single-molecule NP translocation events through a Si_3N_4-NP with a diameter of 40 nm (transmembrane voltage 40 mV). (b) Corresponding signals of λ-DNA single-molecule NP translocation events through a single nanolayer graphene NP with a diameter of 20 nm (transmembrane voltage 140 mV). The current variations are due to the ionic-current blockade and reflect the folding configuration of the translocating DNA.*

3.2 Translocation of Protein-complexed dsDNA with Optical Tweezers (Si_3N_4-NP)

Since we are interested in the quantification of the associated physical mechanisms during molecular translocation, we attached the dsDNA-molecule to a PS-microbead that could be trapped, steered and monitored with our OT setup (Fig. 1b). Upon approaching the NP with a DNA-functionalized microbead and applying a transmembrane voltage (trans: positive) the DNA gets threaded into the NP which can be monitored as a constant force signal. A representative example is depicted in Fig. 4a, where the dsDNA is actively being pulled out of the NP by moving back the microbead with the OT. The jump from the 11 pN background force to zero reflects the threading out of the DNA from the NP. In addition, two characteristic asymmetric force fingerprints (dips) with a retarded force increase extending over more than 200 nm, can be discerned. Each of these asymmetric

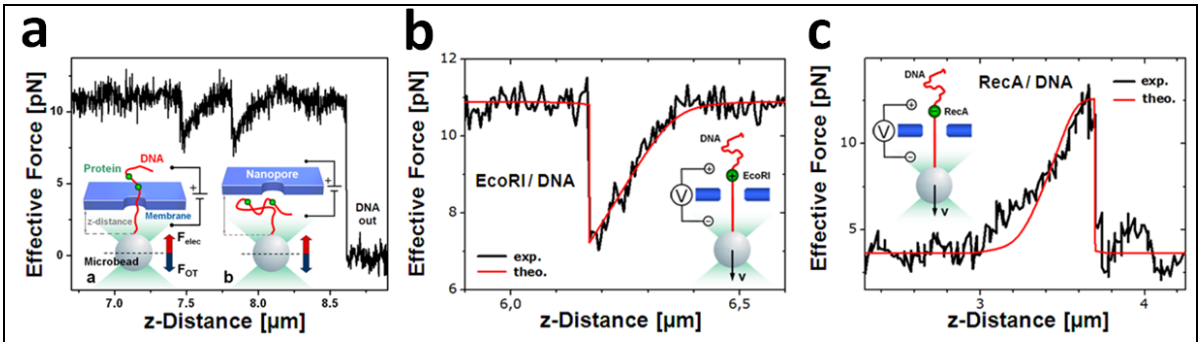

Figure 4 *(a) Force response signal of a protein-complexed λ-DNA-molecule that is being threaded out of a Si_3N_4-NP by OT (45 nm NP diam., 50mV). (b) Close-up of a single-molecule EcoRI/DNA-complex force fingerprint (dip) during NP translocation (45 nm NP diam., 50mV). (c) Force response (peak) of a RecA-complexed λ-DNA-molecule (45 nm NP diam., 20mV). The experimental force response curves (black) in b) and c) were theoretically simulated according a stochastic model[3] (red), yielding the effective protein charge as a result ((b)+59,5e for EcoRI; (c) -700e for RecA-Oligomer) (Figures adapted from Ref. 3).*

force signals can be attributed to a single EcoRI-protein that is translocating through the Si_3N_4-NP (Fig 4b). As we could show in an earlier paper[3], this single protein force signal depends on the effective protein charge. Hence and in contrast to the positively charged EcoRI protein, the force response of negatively charged RecA-proteins leads to an inverted force signal (peak), as it is shown in Fig. 4c.

The experimental force curves could quantitatively be simulated within a theoretical model where an isolated charge on an elastic, polyelectrolyte strand experiences a harmonic nanopore potential during translocation[3]. As a consequence one finds that the total NP potential for a translocating protein (under external mechanical control) exhibits two minima (potential wells) with a barrier (saddle) in between, corresponding to two metastable "states" with the charged protein on either side of the membrane. As the OT moves, the protein translocates through the NP, however, not in a uniform way but with a dynamics that is governed by thermal fluctuations inducing stochastic transitions between

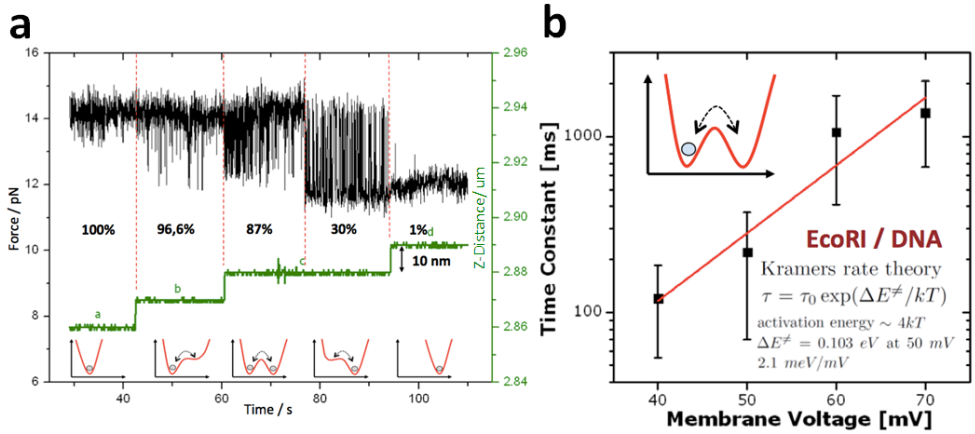

Figure 5 *(a) Thermally activated stochasic transition between the two NP states as can be discerned from the experimental force response curve. The balance between the two-state system can be controlled by the position of the protein along the NP axis coordinate. (b) Estimate of the activation energy between the two NP-states yielding ~4kT at a transmembrane voltage of 50 mV.*

the two states (Fig. 5a). Since these transitions depend on the applied membrane voltage (via the effective NP potential), the measured (averaged) fluctuation time can be related to the activation energy between the two NP states (Kramers rate theory) (see also Fig. 5b).

3.3 Towards Translocation of dsDNA with Optical Tweezers (Graphene-NP)

Since the above physical translocation mechanism is governed by non-equilibrium dynamics and depends on the experimental boundary conditions like protein net charge, transmembrane voltage, NP diameter, membrane thickness, ionic buffer strength, electroosmotic currents through the NP (via NP surface charge) and others, it would be insightful to investigate this phenomenon in more detail with other NP geometries and materials. Therefore we chose graphene for this purpose and prepared single nanolayer graphene which we transferred onto Si_3N_4-membrane supports. The milled graphene NPs with typical diameters of 20-50 nm were tested in Coulter counter experiments (see Figs. 2 and 3), where we monitored individual DNA-passages through the NP.
In contrast, OT-controlled translocation experiments as described in chapter 3.2 for Si_3N_4-NP turned out to be challenging for graphene NP. The reason might be a systematic difficulty, which stems from the fact that a PS-microbead – optically trapped in a strong IR-laser focus - is obviously heavily thermally activated in the presence of a graphene interface (Fig. 6). This observation suggests that at least the glass transition temperature of PS (around 100 °C) is reached, however, no other phenomena (strong convective flow or vapor microbubbles) can additionally be found. Nevertheless, if this assumption would hold, the integrity of PS-microbead, the dsDNA and its biotin-avidin fixation to the microbead would be questionable[9,10].

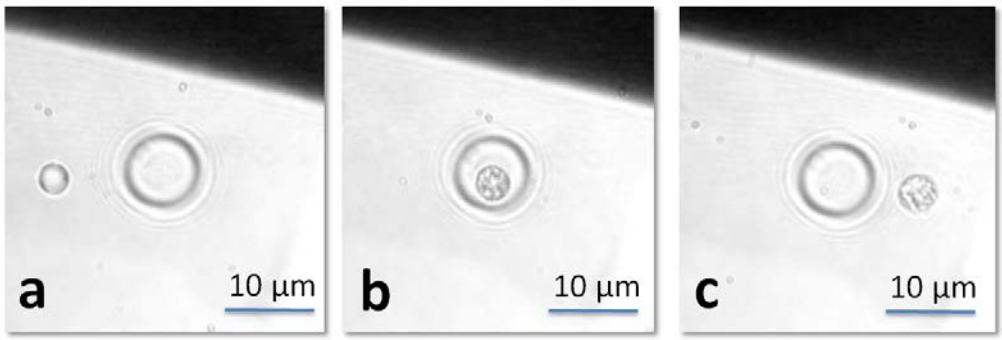

Figure 6 *(a) Video series of light microscopy images, where a PS-microbead (3,28 µm diam.) is positioned with a focussed IR-laser (OT) a few micrometers away from a Si_3N_4-membrane where a single nanolayer graphene is transferred to. The round bore in the middle of the image is a micropore (~10 µm diam.) in the Si_3N_4-membrane window and hosts a free-standing graphene nanolayer (Almost the complete Si_3N_4-membrane is also coated by the graphene). Upon laterally moving the microbead in constant distance across the micropore (with constant laser trapping power of 200 mW), the microbead gets almost instantaneously "heated" only when positioned over the free-standing graphene and turns opaque.*

4 CONCLUSION

The translocation of linear (protein-complexed) λ-DNA through Si_3N_4- and single nanolayer graphene nanopores was investigated with a Coulter counter based setup as well

as with optical tweezers control. For the OT controlled translocation experiments through solid-state Si_3N_4-NP, our results provide convincing evidence that force-controlled translocation dynamics of a polyelectrolyte through a NP is accompanied by a thermally induced, stochastic hopping between two adjacent NP states that can adequately be described by Kramers rate theory. Beyond the possibility to detect and reversibly control the position of a single translocating protein attached to a DNA strand by OT, obviously, the overall elasticity of the DNA-polymer significantly contributes to the retarded force response signals when threaded through a NP. It will be interesting to see how force controlled detection concepts will contribute to single molecule NP-based spectroscopy and analysis in the future.

ACKNOWLEDGEMENTS

We would like to thank Dieter Akemeier, Christoph Pelargus, Helene Schellenberg for technical assistance as well as many stimulating discussions with Cees Dekker, Ulrich Keyser, Armin Gölzhäuser and Andreas Hütten. Financial support from the Collaborative Research Centre 613 of the Deutsche Forschungsgemeinschaft (DFG) is gratefully acknowledged.

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