DNA translocation through nanopores by optical tweezers

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We investigated the translocation mechanisms and dynamics of single dsDNA and dsDNA-protein complexes upon threading through a solid-state nanopore (NP) by quantitative 3D-optical tweezers (OT) (Fig. 1). The combination of OT force mechanics with electrophysiology allows experiments at sub-*pN* force sensitivity, *ms* time resolution and *pA* ionic current sensitivity. In our single molecule translocation experiments, we find distinct asymmetric and retarded force signals that can be associated to individual proteins and depend on the protein charge, the DNA elasticity and its counter-ionic screening in the buffer [1]. A theoretical model where an isolated charge on an elastic, polyelectrolyte strand is experiencing an anharmonic nanopore potential was developed. Its results compare very well with the measured force curves and explain the experimental findings that the force depends linearly on the applied electric field and exhibits a small hysteresis during back and forth translocation cycles. Moreover, the translocation dynamics reflects the stochastic nature of the thermally activated hopping between two adjacent states in the NP that can be adequately described by Kramers rate theory [2].

In our presentation, we will also report on a recent tedious analysis of single DNA translocation experiments where we quantified the effective dsDNA threading force through He-ion-microscope drilled Si_3N_4-solid-state NPs with diameters ranging from 6 nm to 70 nm [3, 4] as well as the working progress of our experiments with biopore toxins.

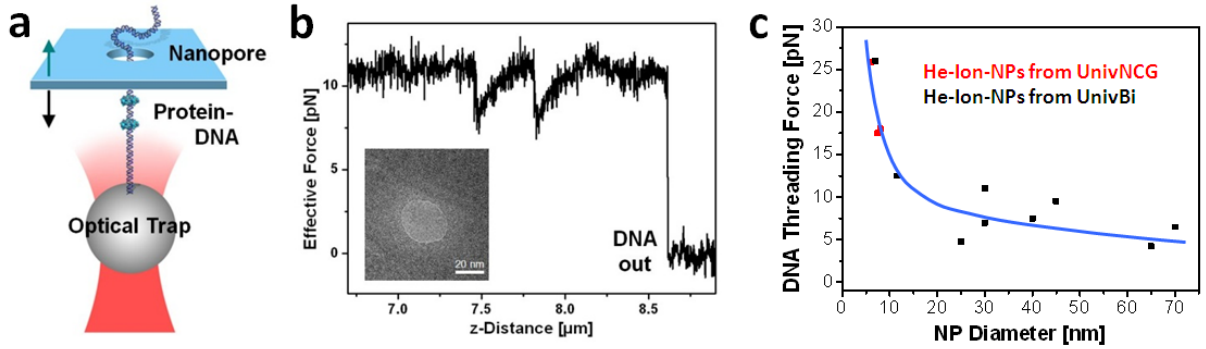

Fig.1. a) Single molecule NP force spectroscopy: a microbead with an attached DNA molecule is optically trapped in the vicinity of a solid-state NP. A membrane voltage drives the negatively charged DNA into the NP. The

electrokinetic forces on the DNA are balanced by the force acting on the bead in the optical trap. The distance between trap and membrane can continuously be varied with nm precision. **b)** In the force-distance curve, two isolated DNA-bound proteins can be detected as distinct signals by unthreading the DNA strand out of the NP. **c)** Dependence of threading force on NP diameter (red dots: NPs fabricated at UNCG, black dots: fabricated at UBi, blue line as *guide to the eye*).

References

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