


P4 – Optical Tweezers Measurements of Threading DNA and DNA-Ligand-Complexes Through Solid-State Nanopores

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We developed a versatile and high precision 3D optical tweezers setup, capable for force measurements completely based on detection of backscattered light with minimal optical interference to measure forces in the sub-pN regime, and to manipulate single molecules. With this novel setup, single dsDNA-molecules were threaded into a solid-state nanopore by applying electrical voltage across the membrane, as the electrostatic force and the ionic current through the pore were measured. Here, individual force steps could be observed for each DNA-molecule entering the nanopore. Active pulling of a single Lambda-DNA-molecule out of the nanopore by linearly increasing the bead-membrane distance induced a distinct force signal, until the DNA was completely pulled out of the nanopore. Binding of dedicated protein ligands (peroxiredoxin, E.coli RNA-polymerase, and RecA) to dsDNA caused a significant change in the apparent electrostatic forces that are required for threading and unthreading the DNA-ligand-complex through the nanopore.



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Single Beam Optical Tweezers with Backscattered Light Detection for threading DNA through Nanopores

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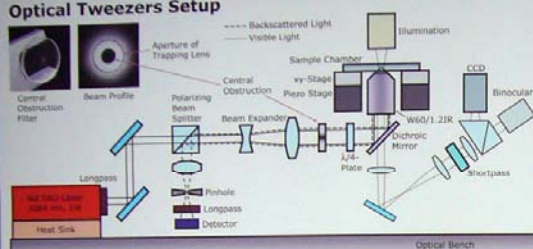
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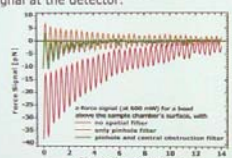
Abstract: We introduce a versatile and high precise optical tweezers setup operated in backscattered light detection mode with minimal optical interference in the vicinity of weak reflective surfaces to measure small forces and manipulate single molecules [1]. Operated with a single IR-laser beam that is spatially filtered by a central obstruction filter, we can perform force measurements in axial direction with remarkably high precision. The setup was tested by threading individual λ -DNA molecules and DNA-protein complexes into a solid state nanopore [2,3], while measuring electrostatic forces and ionic current through this pore.

Optical Tweezers Setup

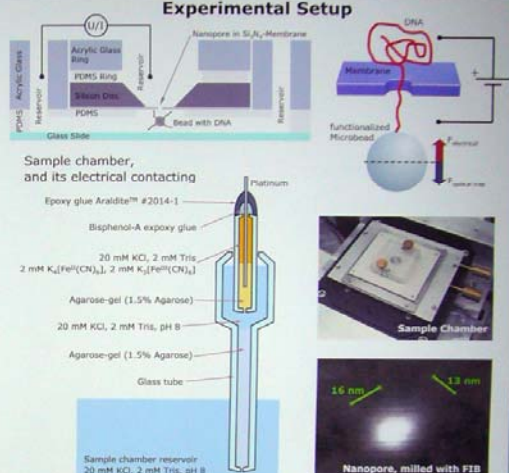


In the vicinity of a weakly reflecting surface (e.g. bottom of sample chamber), backscattered light from that surface interferes with backscattered light from the bead and induces a disturbing interference signal at the detector.

The central obstruction filter (CO) blocks out backscattered light close to the optical axis arising from that surface.



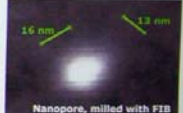
Experimental Setup



Sample chamber, and its electrical contacting

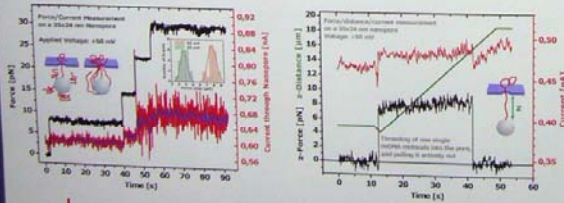
- Epoxy glue Araldite™ #2014-1
- Bisphenol-A epoxy glue
- 20 mM KCl, 2 mM Tris, 2 mM K₂[Fe(CN)₆], 2 mM K₃[Fe(CN)₆]
- Agarose-gel (1.5% Agarose)
- 20 mM KCl, 2 mM Tris, pH 8
- Agarose-gel (1.5% Agarose)
- Glass tube
- Sample chamber reservoir 20 mM KO, 2 mM Tris, pH 8

Nanopore, milled with FIB

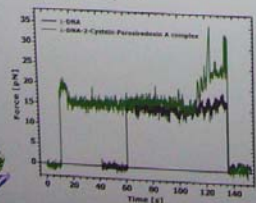


Threading dsDNA and dsDNA-Ligand Complex into a Nanopore


Each DNA-molecule entering the nanopore has simultaneous force and ionic current steps. Increasing the distance between bead and nanopore causes a slightly higher force signal on a 16.4 μ m λ -DNA due to the entropic force, and a simultaneous step of the ionic current signal.



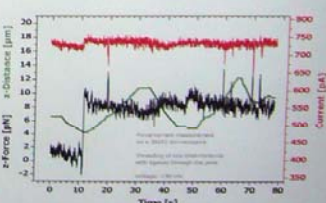
Force response of threaded DNA upon adding groove binding peptide 2-cys-peroxiredoxin, and pulling the complex out of the nanopore.



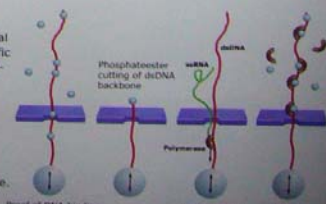
2-Cys-Peroxiredoxin dimer



DNA with DNase threaded into the nanopore, and pulled back and forth. Simultaneous increase of force signal and blocking of ionic current could be observed for each possible bound DNase.



Outlook: Nanopore measurements of dinuclear dsDNA-binding metal-metal ligands, with specific abilities (backbone-cutting, RNAP inhibition). Label-free detection of bound ligands by "scanning" DNA-ligand-complex through the nanopore is possible.



Proof of DNA-binding, DNA-specificity measurement, Proof of RNAP transcription, RNAP inhibition

[1] A. Sischka et al., *Rev. Sci. Instrum.* **79**, 063702 (2008)
 [2] R. M. M. Smeets, C. Dekker et al., *Nano Lett.* **6**, 89 (2006)
 [3] U. F. Keyser et al., *Rev. Sci. Instrum.* **77**, 105105 (2006)

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