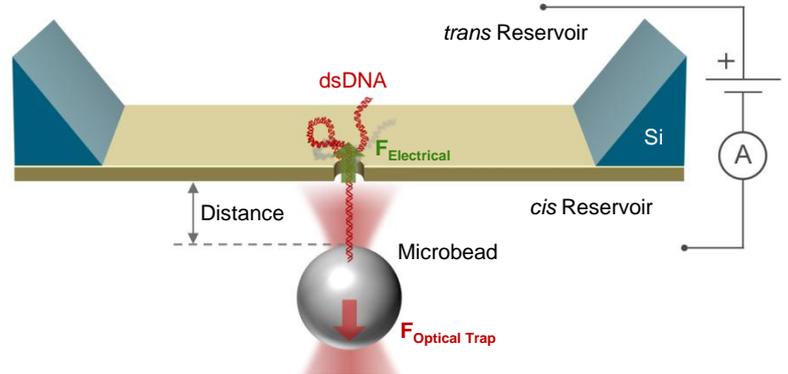
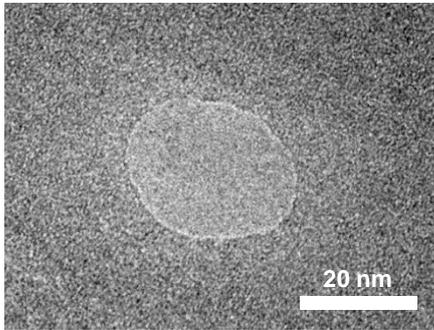




DNA Translocation Through Nanopores

Ionovation PicoTweezers

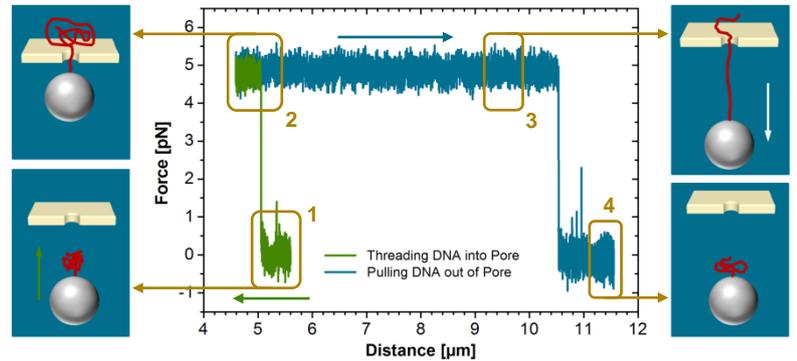
Nanopores play a major role in biology since they have rapidly evolved into a new and promising technique in single-molecule detection. The controlled threading of a single DNA molecule or a DNA-protein complex into a nanopore and the force measurement during this process allows investigation of the translocation dynamics and a localization of the bound protein.



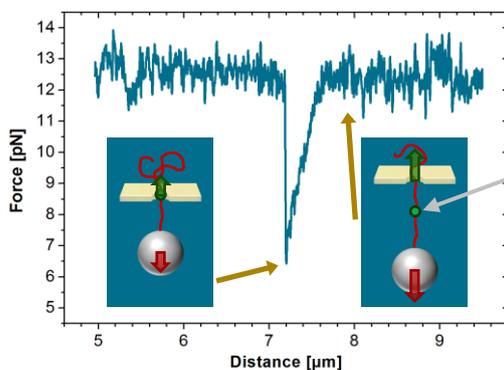
Left: TEM image of a nanopore in a silicon nitride membrane that serves as model system to study single molecule translocations. Right: Experimental setup of a controlled DNA translocation measured with optical tweezers. When applying a voltage across the membrane, a single DNA molecule immobilized on a trapped microbead translocates through the pore. The force acting on the molecule and the distance between bead and nanopore can be precisely measured.

Translocation of a Single DNA Strand

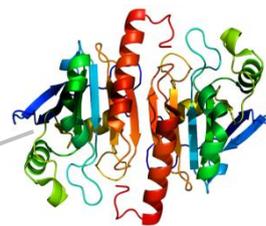
An immobilized DNA is threaded into the pore by electrostatic forces acting on the negatively charged DNA backbone. This effect can be monitored as an abrupt step of the force signal to a certain value, which remains constant even when retracting the bead. The measured force depends on the applied voltage, membrane chemistry and the diameter of the nanopore.



Controlled DNA threading into a 55 nm nanopore with an applied voltage of 50 mV.



Characteristic force signal of a single peroxiredoxin molecule bound to a DNA strand when both are translocated through a 35 nm nanopore. Peroxiredoxin decomposes reactive oxygen species in cells and turning them into cell signaling events.



Single DNA-Bound Protein

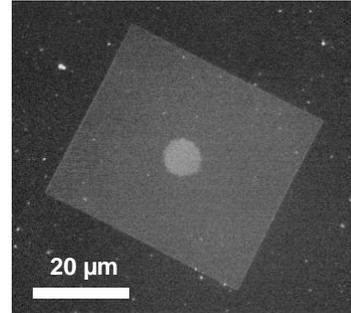
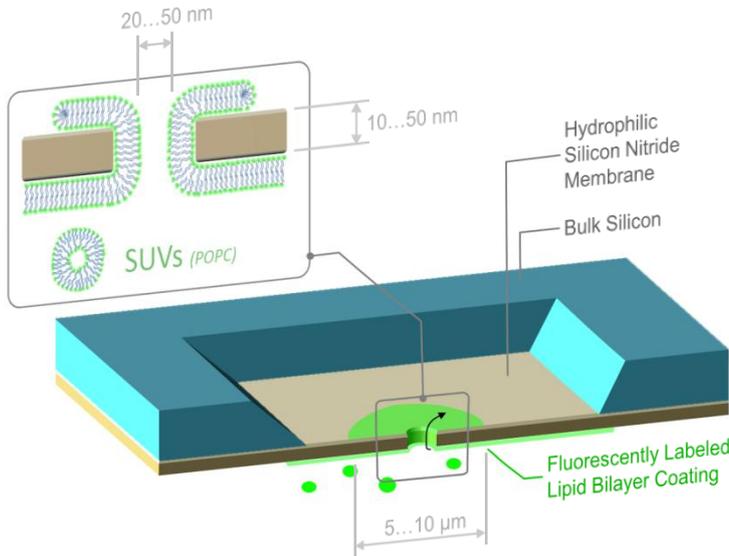
A distinct asymmetric force signal occurs when a ligand (peroxiredoxin) bound to the DNA stand is actively pulled through the pore. Thus, a label-free localization of the protein binding site is possible and yields information on the translocation dynamics.

It can be understood as the result of an effective positive charge of the protein counteracting the negative DNA backbone charge and thus reducing the electrostatic force.

See also: A. Spiering et al., *Nanopore Translocation Dynamics of a Single DNA-Bound Protein*; Nano Letters, **11**, 2978 (2011)



Both the diameter of the nanopore and the membrane material and its surface charge determine the magnitude of the trapping force, which is acting on a single molecule inside the pore. Particularly, the electroosmotic flow through a nanopore can be influenced by coating the pore walls with a lipid bilayer. Lipid bilayer-coated silicon nitride membranes serve as a model system for biological membranes containing nanopores.



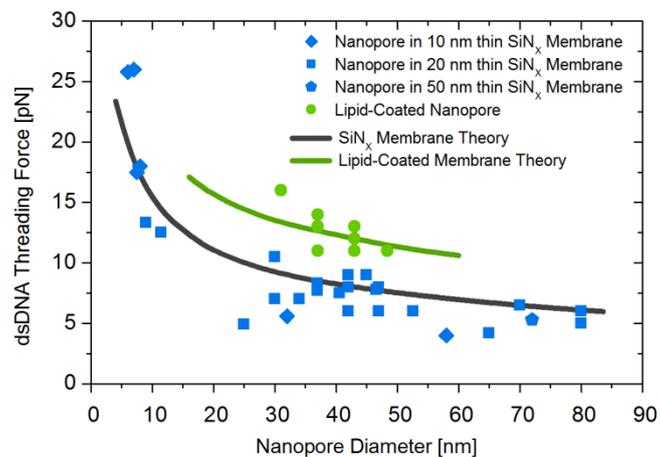
Left: Small unilamellar vesicles (SUVs) get in contact with the silicon nitride membrane, burst, and merge into a lipid bilayer. Right: Fluorescence image of the lipid bilayer completely covering one side of the silicon nitride membrane. The round flake in the center indicates that the lipid bilayer has moved through the nanopore and is now coating the pore wall and the opposite side of the membrane.

A reduced nanopore conductivity compared to the uncoated pore can be electrically monitored, which is primarily caused by pore diameter reduction of about 10 nm after successful bilayer coating of both the membrane and the nanopore wall. This can additionally be confirmed by fluorescence recovery after photo bleaching by adding DOPE labeled with Rhodamine B to the lipid solution before SUV formation.

Threading Force Dependence

Optical tweezers forces measurements on a single dsDNA revealed a strong increase of the threading force upon decreasing the diameter of the pore. This can be attributed to a reduction of the electroosmotic flow in smaller pores, which always opposes the electrostatic force acting on the DNA molecule.

Coating the nanopore walls with an electrically neutral POPC lipid bilayer significantly reduce the electroosmotic flow, too, resulting in an 85 % increased threading force compared to an uncoated pore of the same diameter.



Relation between DNA threading force and nanopore diameter for uncoated and lipid bilayer-coated nanopores at an applied voltage of 50 mV.

See also: L. Galla et al., *Hydrodynamic slip on DNA observed by optical tweezers-controlled translocation experiments with solid-state and lipid-coated nanopores*; Nano Letters, **14**, 4176 (2014)
L. Galla, *Nanopore Modifications with Lipid Bilayer Membranes for Optical Tweezers DNA Force Measurements*; Dissertation, Bielefeld University, Faculty of Physics; April 2015