of alpha-hemolysin. It is argued that channel confinement plays a critical role in dynamics of these complex processes. Our results shed light on the way intermolecular processes affect nucleic acids' kinetics.

2184-Plat
Measuring Direct Forces on dsRNA in Solid State Nanopores
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In recent years, far-reaching discoveries about the functionality of RNA in biology have been made. Especially double stranded RNA (dsRNA) is found to play a key role in the process of RNA interference. We employ solid state nanopores (nanometer sized holes in a thin SiN membrane) to study single RNA molecules. By applying an electrical field over the nanopore, RNA molecules can be threaded into the nanopore, causing a change in the ionic current. This change can provide insight into some of their structural properties, such as charge density, diameter, and possibly also their local structure. We have integrated our nanopore setup with optical tweezers, which allows us to also measure and apply forces to the molecule inside the nanopore.
Here, we present the first application of this new technique to the study of RNA molecules, in this case long dsRNA. We show that the force experienced on these molecules is very similar to that on DNA molecules, as one would expect from the very similar structure of these molecules. In addition, we show that the measured force is independent on the distance of the optical trap to the nanopore, even at very close range (< 500 nm). Measuring forces at such close distances may be required for the application of this technique to more complicated molecules, such as single stranded RNA molecules or RNA-protein complexes. Finally, we have further extended the use of this technique to very small nanopores (down to ~3 nm in diameter), also an important future requirement to study more complex molecules. Combined, these measurements represent important steps towards the detection of local structure along RNA molecules.

2185-Plat
A Pore-Cavity-Pore Nanodevice to TRAP and Electro-Optically Investigate Single Molecules
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Single engineered nanopores in solid state membranes have attracted broad attention in recent years as a tool to study single biological molecules like DNA or proteins. Here we introduce a novel solid-state device which comprises *two* stacked nanopores defining the in- and outlet of a pico liter cavity. This pore-cavity-pore (PCP) architecture allows for the electrical as well as optical examination of single molecules.
The PCP device is fabricated by structuring nanopores into a sandwich SiN/Si/SiN wafer using e-beam lithography, wet chemical etching, and feedback controlled electrochemical etching steps. The in- and outlet nanopores of the fabricated PCP-devices are characterized by transmission electron microscopy, evidencing that the pore diameters may be controlled independently down to 10 nm.
We demonstrate that the double pore geometry enables a novel measurement mode for nanopore devices, namely, time-of-flight experiments. In DNA translocation experiments we find time-correlated pulses in the measured ionic trans-doublepore current, which arise from single DNA molecules translocating one pore after the other. From correlation analysis we are able to deduce molecular mobilities for DNA molecules of different lengths. Moreover, we present fluorescence experiments of single DNA molecules and nm-sized polystyrene beads inside the PCP device. Through electric potential control we are able to inject and eject nano-objects into and out of the PCP device. We utilize fluorescence to monitor hybridiziation of DNA molecules trapped in the cavity in order to demonstrate how the PCP device may be used as a pico liter reaction chamber.

Platform AJ: Protein Aggregates

2186-Plat
Low Resolution Structure of a Membrane-Permeabilizing Oligomer of α-Synuclein: the Basis for a High-Throughput Screening of Compounds against α-Synuclein Aggregation
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α-synuclein is a 140-residue natively unfolded protein, whose aggregation is implicated in the development of Parkinson's Disease. It is thought that the cytotoxic species is not the mature fibril, but rather a prefibrillar aggregate which has membrane-permeabilizing properties. We have used Small Angle X-ray Scattering (SAXS) to determine the low-resolution structures of the different species formed during α-synuclein fibrillation in a non-invasive fashion. In addition to the starting monomer-dimer equilibrium and two *bona fide* fibril types accumulating towards the end of the aggregation process, we have identified a wreath-shaped oligomeric state which has a very distinct central hole. Both its structure and the kinetics of its formation are consistent with an on-pathway role, while its membrane-permeabilizing properties identify it as a putative cytotoxic species. We have also used SAXS to monitor the fibrillation of α-synuclein in the presence of the surfactant SDS and find that the fibrillar aggregates grow in a continuous fashion, forming beads on a string where the individual beads are stabilized by intermolecular α-synuclein contacts. The high reproducibility of this aggregative behaviour has formed the basis for a high-throughput screening assay involving 746.000 compounds that has allowed us to identify a significant number of compounds with the ability to inhibit early-stage aggregation of α-synuclein. This distinguishes the assay from previous assays that have focused mainly on the ability to prevent formation of α-synuclein fibrils. The hits from our assay may form the basis for a therapeutic intervention against Parkinson's Disease.

2187-Plat
Molecular Insights into the Role of Serum Amyloid-P Component in the Stabilzation of Fibrillar Beta-2 Microglobulin
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Serum amyloid-P component is found ubiquitously in amyloid deposits and has been shown to stabilize fibrillar structures and prevent clearance by the host's defences. Here we report on solid-state NMR studies on fibrillar deposits formed from β2-microglobulin, typically found in patients with dialysis related amyloidosis, and their interactions with serum amyloid-P component.
We have successfully undertaken the expression, purification and refolding of the 99 residue β2-microglobulin and established conditions for optimal binding of serum amyloid-P component. High resolution solid-state magic-angle spinning (MAS)-NMR spectra obtained from the fibrils indicate that within the fibrils the β2-microglobulin adopts a homogeneous structure. Using two-dimension homo- and hetero-nuclear correlation spectroscopy we have been able to assign several of the sites within the protein. Currently we are using a range of labelling schemes and acquiring three-dimensional data-sets to complete this assignment. Comparison of the assignment with that obtained from monomeric β2-microglobulin in solution is beginning to provide valuable insights into the structural changes occurring upon fibrilization. Similar comparisons with fibres decorated with serum amyloid-P component should provide valuable insights into how this molecule interacts and stabilizes amyloid fibrils at a molecular level.
During the course of these experiments we have also obtained 2D correlation data on inclusion bodies of β2-microglobulin. The resolution attained is not as high as that observed in the fibrillar spectra, however they permit the assignment of resonances to amino acids with the β2-microglobulin. This suggests that within the inclusion bodies the β2-microglobulin adopts a well defined conformation with the lower spectral resolution arising from reduction in dynamics in these highly packed structures. Detailed comparisons of the data with that obtained from the soluble and fibrillar β2-microglobulin should provide insights into the nature of this structure.

2188-Plat
The Role of Small Oligomers on an Amyloidogenic Free Energy Landscape
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We combine atomic force microscopy particle size distribution measurements with earlier measurements on 1-anilino-8-naphthalene sulfonate, thioflavin T and dynamic light scattering to develop a quantitative kinetic model for the aggregation of beta-lactoglobulin into amyloid. We directly compare our simulations to the population distributions provided by dynamic light scattering and atomic force microscopy. We combine species in the simulation according to structural type to compare with the fluorescence fingerprint results. The kinetic model of