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**
**Michiel van den Hout**, Igor D. Vilfan, Susanne Hage, Serge Donkers, Aartjan te Veldhuis, Nynke H. Dekker.
Delft University of Technology, Delft, Netherlands.
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.

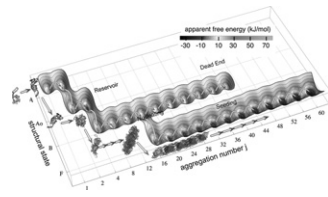
**2185-Plat**
**A Pore-Cavity-Pore Nanodevice to TRAP and Electro-Optically Investigate Single Molecules**
**Daniel Pedone**, Martin Langecker, Ulrich Rant.
Technische Universität München, Garching, Germany.
Single engineered nanopores in solid state membranes have attracted broad attention in recent 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**
Lise Giehm[1], Bente Vestergaard[2], Cristiano Oliveira[1], Jan S. Pedersen[1], Dmitri I. Svergun[3], Keith Pitts[4], Girija Krishnamurthy[4], Roland Staal[4], Peter Reinhart[4], **Daniel E. Otzen[1]**.
[1]University of Aarhus, Aarhus C, Denmark, [2]Copenhagen University, Copenhagen, Denmark, [3]EMBL Hamburg Outstation, Hamburg, Germany, [4]Wyeth Ltd., Princeton, NJ, USA.
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**
Garrick Taylor[1], Joern M. Werner[1], Steve P. Wood[2], **Philip T.F. Williamson[1]**.
[1]University of Southampton, Southampton, United Kingdom, [2]Royal Free Hospital, UCL, London, United Kingdom.
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**
**David S. Talaga**.
Rutgers, The State University of New Jersey, Piscataway, NJ, USA.
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



amyloidogenesis leads to an aggregation free energy landscape. We define the roles of and propose a classification scheme for different oligomeric species based on their location on the aggregation free energy landscape. We relate the different types of oligomers to the amyloid cascade hypothesis and the toxic oligomer hypothesis for amyloid-related diseases. We discuss existing kinetic mechanisms in terms of the different types of oligomers. We provide a possible resolution to the toxic oligomer-amyloid coincidence.

**2189-Plat**
**Amyloid-Like Cross-Beta Structure Polymorphism: An Energetic Point of View**
**Xavier Periole**[1], Thomas Huber[2], Thomas P. Sakmar[2], Siewert-Jan Marrink[1].
[1]University of Groningen, Groningen, Netherlands, [2]Rockefeller University, New York, NY, USA.
Amyloid fibrils are highly ordered protein aggregates involved in numerous pathological conditions, including neurodegenerative diseases. A single mature unbranched fibril is formed from at least several interacting protofilaments, which share a common structural feature - a cross-β spine, in which β-sheets are aligned with the fibril's main axis. It has been observed that amyloid fibrils may exist with different morphologies and twists depending on their mode of preparation, even within a single sample. However, the precise etiology and pathological implications of such twist-polymorphism are unclear. We present here the results of a series of molecular dynamics (MD) simulations of a protofilament model formed by 40 copies of the GNNQQNY peptide fragment of the yeast prion protein, Sup 35. The planar protofilament observed in the crystal structure displays no free energy barrier against twisting in the absence of crystal packing interactions. Umbrella sampling simulations, in which the twist between consecutive peptides is used to control the overall protofilament's twist, confirm that the free energy minimum is observed at a 7.5 degree left-handed twist conformation. There is little apparent free energy penalty derived from twisting the cross-β structure in the range of −12 to 0 degrees. Moreover, the twist of the cross-β structure is enthalpy-driven, and while the backbone favors the straight form of the protofilament, side chains favor the twisted form. We propose that the twist of a protofilament might easily adapt to external stresses such as interactions with other protofilaments. This hypothesis is further illustrated by our characterization of different morphologies of protofilament assemblies composed of one to four protofilaments. Taken together the data support an energetic basis for the different twist-morphology states observed in amyloid fibrils.

**2190-Plat**
**Dissecting the Membrane Dynamics of Amyloid Oligomers at a Single Molecule Level**
**Martino Calamai**[1], Martin Zanni[2], Francesco Pavone[1].
[1]LENS - European Laboratory for Non-Linear Spectroscopy, University of Florence, Sesto-Fiorentino (Florence), Italy, [2]Department of Chemistry, University of Wisconsin, Madison, WI, USA.
Fibrillar deposits of proteins are the hallmark of amyloid diseases, amongst which Alzheimer's disease stands out as the most widespread neurodegenerative pathology of the brain. Neuronal dysfunction is currently attributed to the interaction of A-beta oligomers with the plasma membrane. Several scenarios have been proposed, but the mechanisms of binding of the oligomers to the cell membrane and their subsequent toxicity is still unclear. Distinct results indicate that oligomers may insert non-specifically into the lipid bilayer, or bind to specific targets, such as post-synaptic structures or gangliosides characteristic of lipid rafts. In general, these studies have investigated the averaged features of an ensemble of molecules.
Here, we have been able to successfully monitor the mobility of single A-beta oligomers on the plasmamembrane of living neuroblastoma cells. Preformed oligomers were incubated with cells and subsequently labelled with monoclonal primary antibodies and secondary Fab fragments coupled to quantum dots (QDs). Single QDs bound to the oligomers were then tracked.
The analysis of the trajectories reveals that most of the oligomers show a highly confined membrane mobility, suggesting a potential involvement of the cytoskeleton, while some diffuse laterally following a free Brownian motion. Strikingly, we found that other amyloid aggregates sharing a similar conformational structure but composed of different proteins (amylin and prion Sup35) display comparable dynamics. Moreover, we discovered that the presence of amyloid aggregates decreases dramatically the membrane diffusion of GM1 gangliosides labelled with biotinilated cholera toxin coupled to streptavidin-QDs.
Overall, these results enable a better understanding of the basic mechanisms underlying Alzheimer's diseases and other amyloid pathologies.

**2191-Plat**
**Physical Properties of Yeast Prion Proteins Studied with Optical Tweezers**
**Carlos E. Castro**, Jijun Dong, Mary C. Boyce, Susan Lindquist, Matthew J. Lang.
MIT, Cambridge, MA, USA.
Formation of amyloid fibers plays a vital role in both natural biological processes and neurodegenerative disease. Recently, amyloid formation has been shown to be a general property of proteins and peptides. Their impressive mechanical properties, which are comparable to spider silk, combined with their ease of assembly in synthetic preparations make amyloid fibers particularly suited for nanomaterials applications, including as templates for conducting nanowire formation, as scaffolds for cell growth, and as functionalized biosensors. Prion proteins are a special class of amyloid fiber forming proteins which are self-templating and thereby transmissible as disease vectors. This work combines optical tweezers force spectroscopy with fluorescence imaging to study the physical properties of amyloid fibers formed from polymorphic variants of a 253 amino acid N-terminal fragment (NM) of the yeast prion protein Sup35. Experiments revealed that fibers associated with a ''weak'' NM prion strain have an approximately 2-fold larger bending stiffness than those associated with a ''strong'' NM prion strain. We further subjected NM fibers to multiple cycles of forces up to 250 pN resulting in unfolding of individual prion subdomains and rupture of intermolecular interactions. Our results have implications for the physical basis of prion strain diversity and give important insights into the underlying structure of Sup35 prions.

**2192-Plat**
**Probing the Conformational Ensemble of Polyglutamine During the Initial Stages of Aggregation**
**Scott Crick**, Rohit V. Pappu.
Washington University, St. Louis, MO, USA.
Nine different neurodegenerative diseases, including Huntington's disease, are associated with the aggregation of proteins whose only commonality is a repeating stretch of glutamine. Experiments and computer simulations have demonstrated that monomeric forms of polyglutamine molecules sample heterogeneous sets of collapsed structures in water. Molecular simulations have predicted that these molecules spontaneously associate at conditions approaching those of typical in vitro experiments for chains of length N>15. Moreover, the spontaneity of these homotypic associations increases with increasing chain length. These results suggest that polyglutamine aggregation is unlikely to follow a homogeneous nucleation mechanism, which is currently the most widely accepted mechanism by which polyglutamine aggregation is thought to occur. In this work, we test these predictions using both steady state and time resolved Förster Resonance Energy Transfer (FRET). Hopefully this work, along with the simulation results, will allow a better understanding of how monomeric polyglutamine assembles into soluble oligomers and, eventually, insoluble aggregates.

**2193-Plat**
**Changing the Kinetics of Amyloid Beta Plaques Formation: Implications for Alzheimer's Disease Immunotherapy**
**Jeffy P. Jimenez**[1], Maj-Linda Selenica[1], David Morgan[1], John Lin[2], Norma A. Alcantar[1].
[1]University of South Florida, Tampa, FL, USA, [2]Rinat-Pfizer, San Francisco Bay Area, CA, USA.
Clear evidence exists linking the presence of neuritic amyloid beta (Aß) peptides plaques with the brain's tissue deterioration and cognitive impairment in patients with Alzheimer's disease (AD). Removing these plaques results a logic approach to treat patients with AD. Understanding the plaque formation mechanisms is key to developing strategies to remove them. In previous studies our group has investigated the plaque formation process using attenuated total reflection Fourier infrared (ATR-FTIR) spectroscopy and atomic force microscopy (AFM), observing how different Aß peptide aggregates formed and under what kinetic conditions they assembling into mature fibrils. We have been able to determine the changes in the secondary structure of the peptide molecules during this process. In this work, we combined the same analytical techniques to investigate the use of different anti-Aß monoclonal antibodies to analyze the process of destabilization and prevention of AD plaque formation. We compared the changes of kinetic rates of fibrillization when the peptides were incubated with different antibodies from the early stage of aggregation, at pH 7.4 and 37°C. The molar ratio of antibodies to peptide used was 1:1000. We found that some antibodies considerable decrease the formation of parallel beta sheets structures increasing the formation of alpha helix structures or unordered,