the host cells. This represents the first step of infection and requires multiple simultaneous interactions since the affinity between one single HA-SA pair is very low (10-13 M-1).
The binding interaction of influenza virus adhesion to living cells was probed by means of dynamic force spectroscopy and force probe molecular dynamics (MD) simulation. We applied three independent approaches to measure the unbinding force between influenza virus and a host cell membrane. Using optical tweezers and AFM based single molecule force spectroscopy we were able to characterize the binding energy on the single molecule level. Unbinding events where analysed and revealed a multimodal rupture force distribution which suggests sequential binding of multiple receptors. We determined the interacting force between hemagglutinin and its receptor sialic acid to be ~10pN. Furthermore we used molecular dynamics simulation to gain information about the binding architecture and the sequence of the unbinding process. MD simulation allowed us a more detailed view of the energy landscape that governs the interaction between HA and its ligand.
The combination of experimental and simulated force spectroscopy covers a very large force regime and provides information that could not be obtained with either one or the other method. The techniques are complementary and provide detailed insights of the molecular interactions involved in influenza virus attachment.

119-Plat
Mechanical Mapping of Single Proteins at Subnanometer Resolution using AFM
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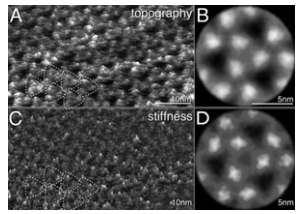

The structure and function of proteins are determined by their mechanical stability. The temperature- or B-factors obtained from crystallographic data provide an indirect measure of the order and stability of the protein, together with structural information. However, B-factors from crystallography analysis may be related to the protein packing in the 3D-crystal and are not quantitative. A direct quantification of the mechanical stability of proteins based on atomic force microscopy (AFM) consists in mechanically unfolding individual proteins by pulling from two points. However, its correlation with protein structure requires additional measurements. In this work we apply a novel AFM imaging mode based on force spectroscopy (PeakForce) to simultaneously acquire structural and mechanical information of membrane proteins. We used the well-known protein bacteriorhodopsin from *Halobaterium salinarum* as a model system, obtaining topographical and stiffness maps with subnanometer resolution. The characteristic trimeric organization of the trigonally packed (a = b = 62.5Å; γ= 120°) bacteriorhodopsin was clearly visible with inter-monomer distances of 3.2 nm. Overlay of stiffness and topography maps allowed the investigation of nanomechanical properties at the single molecule level providing a map of the structural stability of bacteriorhodopsin.

120-Plat
Force Spectroscopy of Mammalian Prion Protein Folding and Misfolding
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Prions are unconventional infectious agents that have the capacity to trigger the native prion proteins (PrP^{C}) to adopt a misfolded conformation (PrP^{Sc}) which leads to aggregation and neurodegenerative disease. Although the mechanism of PrP^{Sc} formation is still unknown, partially-folded intermediate or misfolded states are thought to play important roles in the conversion and amyloid formation processes. Here, we study the folding of the prion protein by single molecule force spectroscopy using optical tweezers and present the first direct observation of partially structured misfolded states. Single molecules of recombinant mammalian prion protein held under tension are repeatedly unfolded and refolded, revealing that the prion protein folds and unfolds cooperatively as a two-state system, with no observable on-pathway intermediates. Intriguingly, we observe multiple off-pathway misfolded states with different lifetimes and extensions. We explore the thermodynamics and kinetics of these states and characterize the different folding pathways of the protein.

121-Plat
DNA Unwinding Mechanism of a RecQ Helicase from Arabidopsis Thaliana Investigated with Magnetic Tweezers
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Helicases are ATP hydrolysis driven molecular motors that processively unwind double stranded DNA. In this study we investigate RecQ2 helicase from Arabidopsis thaliana (AtRecQ2) which plays an important role in genomic maintenance. We use high resolution magnetic tweezers in order to probe the unwinding of a DNA hairpin by this enzyme in real time under different external forces. We find an unwinding rate of 7-9 bp/s which is slow compared to many prokaryotic helicases. Applied forces between 5 and 12 pN only weakly affect this parameter, while the AT versus GC content of the unwound DNA has a significant impact. The weak force- but the relatively strong sequence dependence of DNA unwinding is in disagreement with a passive ratchet unwinding mechanism. High-resolution measurements reveal that AtRecQ2 unwinds the DNA in 3-4 bp steps. Beyond the behavior of AtRecQ2 during unwinding we analyze its behavior on single-strand DNA. While failing to detect single-strand DNA translocation, the data in contrary suggests that AtRecQ2 diffuses on single stranded DNA. Such a weak contact to single-strand DNA is supported by the observation that even on a stretched hairpin configuration the enzyme is capable of repetitive shuffling, i.e. the instantaneous restart of DNA unwinding after a terminated event. Based on this we hypothesize that AtRecQ2 switches between a processive unwinding-mode on double-stranded DNA and a diffusive-mode on single-stranded DNA in order to keep a target hairpin constantly open or to search for a new distant target fork.

122-Plat
Massively Parallel Single-Molecule Manipulation using Centrifugal Force
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Precise manipulation of single molecules has already led to remarkable insights in physics, chemistry, biology, and medicine. However, two issues that have impeded the widespread adoption of single-molecule techniques are equipment cost and the laborious nature of making measurements one molecule at a time. To meet these challenges, we have developed an approach that enables massively parallel single-molecule force measurements using centrifugal force [1]. This approach is realized in an instrument that we call the centrifuge force microscope in which objects in an orbiting sample are subjected to a calibration-free, macroscopically uniform force-field while their micro-to-nanoscopic motions are observed. We demonstrate high-throughput single-molecule force spectroscopy with this technique by performing thousands of rupture experiments in parallel, characterizing force-dependent unbinding kinetics of an antibody-antigen pair in minutes rather than days. Additionally, we verify the force accuracy of the instrument by measuring the well-established DNA overstretching transition at 66 +/- 3 pN. Currently, we are taking steps to integrate high-resolution detection, fluorescence, temperature control and a greater dynamic range in force. With significant benefits in efficiency, cost, simplicity, and versatility, single-molecule centrifugation has the potential to expand single-molecule experimentation to a wider range of researchers and experimental systems.
[1] K. Halvorsen, W.P. Wong, Biophysical Journal - Letters 98 (11), (2010).

123-Plat
Freely Orbiting Magnetic Tweezers: A New Twist on Single Molecule Force Spectroscopy
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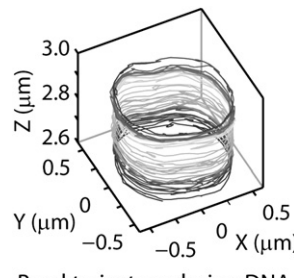
TU Delft, Delft, Netherlands.
A wide variety of cellular processes, including replication, transcription, and recombination, alter the linking number of DNA. Understanding such processes therefore requires measuring rotation; however, it has only recently become possible to make such measurements directly at the single molecule scale. We have developed a new instrument, the freely orbiting magnetic tweezer (FOMT), which permits straightforward, direct measurement of rotary motion in biopolymers.


Bead trajectory during DNA unwinding by RecA.
We demonstrate that FOMT can observe rotation of a dsDNA tether simply by

tracking the trajectory of an unlabeled, commercially available, superparamagnetic bead. We show that no torque is applied to the system by the magnet, facilitating unbiased studies of processes that untwist dsDNA over many turns. Further, we demonstrate that, similar to a conventional magnetic tweezer, controlled forces may be applied to the complex under study.
We also discuss results from experiments employing FOMT, including including measurements of the torsional persistence length of DNA in the 0 - 2.5 pN force range, and direct observation of DNA unwinding during RecA filament formation.

PLATFORM J: Ligand-gated Channels

124-Plat
Studies of the Mechanism of AMPA Receptor Activation using Disulfide Trapping
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Ionotropic glutamate receptors are the major mediators of excitatory synaptic transmission in the vertebrate CNS. Many questions regarding the mechanism of channel activation remain unanswered. In particular, the specific structural trigger within the ligand-binding domain (LBD) that activates the channel portion of the protein is not clear. The bilobed LBD must close to activate the channel, but crystal structures of partial agonists, such as kainate, show only partial lobe closure. The question is whether all compounds that activate the channel can fully close the lobes, and if this fully closed conformation is necessary for channel activation. To begin to address this, dual cysteine point mutations were designed with the potential to lock the lobes in a fully closed conformation when oxidized to form a disulfide bond. Crystal structures and NMR spectroscopy showed that, when bound to glutamate, the disulfide bond can be formed and the lobes locked in a closed state. In addition to glutamate, crystal structures have demonstrated that the disulfide bond can be formed when the partial agonists, kainate and iodowillardiine, are bound to the LBD. These results are supported by chemical shift changes in NMR experiments in oxidizing vs. reducing conditions. Furthermore, with NMR, we have shown that the lobes can be locked closed in the presence of CNQX but not DNQX and UBP282. DNQX and UBP282 are antagonists that are sufficiently large to prevent the lobes from closing and the disulfide bond from forming. CNQX is classically considered an antagonist, but can act, in some cases, as a partial agonist. Taken together the findings suggest that even very weak partial agonists can assume a fully closed state in solution, and support the hypothesis that a fully closed conformation is required to activate the channel.

125-Plat
Structure & Mechanisms Underlying Heteromeric Glutamate Receptor Assembly
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Ionotropic glutamate receptors (iGluRs) are widely distributed in the CNS and play important roles in regulating synaptic transmission and neuronal excitability. The large extracellular Amino Terminal Domain (ATD) regulates the subtype specific assembly of heteromeric iGluRs but the molecular mechanisms controlling assembly are not well understood. Kainate receptor GluK1-GluK3 subunits form functional homomeric ion channels while GluK4 and GluK5, previously named KA1 and KA2, are obligate heterotetramers encoded by a different gene family with only 40% identity to GluK1-GluK3. Using analytical ultracentrifugation we have determined that ATDs of GluK2 and GluK5 form high affinity heterodimers, with a Kd of 77 nM, 5500-fold lower than the Kd for GluK5 homodimer formation. Further, we solved crystal structures for GluK2/GluK5 heterodimer assemblies. Our structures reveal that the two protomers in GluK2/GluK5 heterodimers assemble in a similar arrangement to that observed in the GluK2 ATD homodimer with a pseudo two-fold axis of molecular symmetry. We also solved the structure of a GluK2/GluK5 ATD heterotetramer which reveals that GluK2 mediates the dimer of dimers interactions. The results of chemical crosslinking experiments in full-length heteromeric GluK2/GluK5 match that observed in the ATD crystal structure. The GluK2/GluK5 ATD heteromeric structures along with the GluK2 & GluK5 homodimer structures identify for the first time the elements of heteromeric iGluR recognition & suggest a mechanism for heteromeric assembly in the intact KARs.

126-Plat
Subunit Specific Impact of *Lurcher* Motif Residues on NMDA Receptor Gating
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In all glutamate-activated channels, the outward portion of the M3 transmembrane helix consists of a conserved nine amino acid residue stretch (*lurcher* motif): SYTANALAAF. Substitution of position 8 alanine with threonine (A8T, the *lurcher* position) in a wide range of glutamate receptors result in constitutively active channels. Scanning mutagenesis and cysteine modification experiments, implicated the position 7 alanine (A7) of GluN1 and GluN2 subunits as critical elements in gating, perhaps the equivalent of the 'bundle crossing' in potassium-selective channels. To investigate how A7 and A8 residues affect NMDA receptor gating, we used single channel recordings and kinetic models of receptor activity from HEK 293 cells. We found that substitutions at these positions in GluN1 or GluN2A resulted indeed in channels with modified gating. Compared to wild-type receptors ($P_O = 0.51 \pm 0.05$, MOT = 8.6 ± 1 ms, MCT = 7.5 ± 0.7 ms), A7C and A8T resulted in distinct gating patterns depending on the subunit in which they were introduced. GluN1(A7C) had shorter openings ($P_o = 0.02 \pm 0.01$, MOT= 3.2 ± 0.5 ms, MCT = 143 ± 17 ms) but GluN2A A7C) had significantly prolonged openings ($P_O = 0.17 \pm 0.02$, MOT = 13 ± 1 ms, MCT = 65 ± 7 ms). Further, A8T prolonged openings in GluN1 ($P_O = 0.41 \pm 0.07$, MOT = 21 ± 4 ms, MCT = 30 ± 6.4 ms) but had no effect on gating when introduced in GluN2A ($P_O = 0.57 \pm 0.08$, MOT = 11.2 ± 1 ms, MCT = 9.5 ± 2.6 ms). The implication is that the A7 and A8 positions play distinct gating roles in GluN1 vs. GluN2A subunits, supporting the view that in NMDA receptors, subunits are staggered along M3 into the channel's external vestibule.

127-Plat
A Hydrophobic Bundle Crossing Found in Glutamate Receptor and K+ channel Pore Helices Dominates the Conformational Free Energy Landscape
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A conserved sequence of hydrophobic residues in ionotropic glutamate receptors is believed to be important for determining the channel's resting state. This sequence is homologous in many K+ channel helices, specifically the KcsA pore lining M2, for which a pH sensor controls channel gating. The mechanism of channel gating in KcsA remains poorly characterized. Umbrella sampling, a method to compute free energy differences based on MD simulations, is used to investigate the change in free energy required to transition from a closed to an open KcsA channel conformation. Physical mechanisms that control this transition are analyzed in detail using computational mutagenesis. A potential of mean force is constructed within a two-dimensional reaction coordinate between opposing helices in the M2 transmembrane region of KcsA. The M2 bundle crossing acts as a hydrophobic gate, providing 4 kcal/mol to the total 5.3 kcal/mol free energy barrier between a closed and open conformation. Two of the three known pH sensor residues E118 and E120 are important for stabilizing the intracellular region of the M2 bundle while the third, H25 on M1, initiates disruption of the hydrophobic gate initiating channel opening. Arginine residues within the charged intracellular region of M2 are also shown to stabilize the M2 bundle although do not drive M2 into an open channel conformation. Water density fluctuations at the hydrophobic gate boundary show vapor-liquid phase transitions as the channel pore opens. It is hypothesized that the M2 hydrophobic bundle crossing within KcsA provides a barrier to ion conduction through hydrophobic forces excluding water. H25 protonation provides the energy to disrupt the M2 hydrophobic gate inducing a local water phase transition. Hydration or wetting of the pore creates an open conducting ion channel.

128-Plat
Optogenetic $GABA_A$ Receptors: Controlling Neural Inhibition with Light
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$GABA_A$ receptors are the major mediators of inhibitory neurotransmission in the mammalian brain. GABAergic transmission is highly heterogeneous *in vivo* due to the diverse receptor subunit composition and localization. To better elucidate the roles of different $GABA_A$ receptors in the neural signaling network, it is desirable to develop tools to modulate the activity of a specific