

was performed. Correlated imaging and high resolution mapping allowed unprecedented insights into dynamic membrane structure and behaviour.

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Biophysics of the Hyaluronan-Rich Pericellular Coat

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The articular cartilage consists of a complex extracellular matrix (ECM), which is subject to a high mechanical loading. To counteract the ongoing abrasion, a specialized cell type is embedded within the ECM. These so called chondrocytes constantly renew the ECM. To live and even divide in such a mechanically challenging environment, chondrocytes are protected by a several micron thick pericellular coat (PCC). This coat is of vital biological importance for example in cell proliferation and migration, but also in diseases like osteoarthritis or with age.

The PCC consists mainly of water. Thus it remains invisible in all light microscopy techniques. Where the individual components and even their molecular interactions are well understood, much less is known about the mesoscopic structure of the PCC, which in turn is vital to understand force transductions.

In order to analyze the molecular architecture of the PCC, we established a new set of tools to measure and manipulate the PCC on living cells. The micromechanical profile was measured with position-sensitive passive microrheology. In contrast to other mechanical techniques applied to the PCC, it is independent of the adjacent cell body and enables the measurement in the z direction normal to the cell surface. The observed profiles show a decreasing viscoelasticity within the PCC correlating to a decreasing concentration of the polymer backbone (hyaluronan).

Further, we demonstrate that the PCC expression depends on the cell's interactions with the ECM. In order to precisely control adhesion, we employed nanostructured surfaces. Chondrocytes not only change their adhesion areas in respect to the ligand density, but also the size of their PCC. The modified PCC size could also be related to changes in its mechanical properties analyzed in ongoing experiments.

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Functional Mapping of Single Molecules and Gels using Atomic Force Microscopy

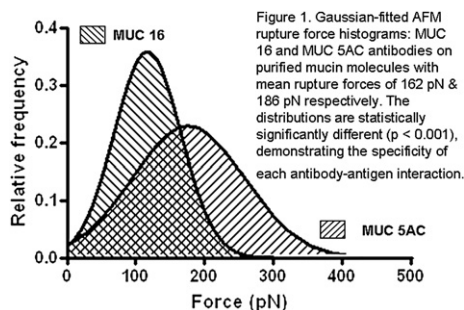
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We have used Atomic Force Microscopy (AFM) to map the spatial distribution of epitopes in the peptide core of mucin glycoconjugates, and also their glycosylation. Using AFM in a physiological environment, and tips functionalised with antibodies against MUC-gene specific epitopes in the peptide core, and also with lectins which recognise terminal sugars, we mapped both single molecules and complex macromolecular structures.

From the simultaneous topographic and force-spectroscopy data, the force-volume maps, we directly quantified the localization, number, and rupture force of recognition bonds on different epitopes of mucin molecules (Figure 1). The specificity of the interactions was confirmed using antibody-blocking peptides, and blocking sugars, and as expected, we observed a loading rate dependence of the unbinding strength.

We have extended these single molecule measurements to more complex physiological gel systems, such as the soft gel layer of the tear film. Binding experiments confirmed the relative abundance of two sugars, previously established by chemical analysis of purified mucins. This comprehensive approach to single molecules and their macromolecular assembly provides an insight into extracellular configuration and packaging of mucins in a gel, and points towards new applications of AFM force mapping.



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Electroporetic Identification Of Cancer Cells By Afm And Fluorescence Techniques

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We carried out a comparative study of cancer cells and normal cells by AFM and found that there exists a sufficient electroporetic difference between them. We measured the population and size of the "bulges" on cell surface, possibly indicating a step directly preceding electroporation, and of the pores upon repeatedly applying an electric field (0 ~ 2 kV). We found that the bulge population of the cancer cell was much higher than that of the normal cell at low electric potentials (0 ~ 500 V), while the pore size of the cancer cell was much bigger by several times. This phenomenon is believed to be due to the elastic difference of the cell membrane, as is actually confirmed by our force measurement that the cancer cell was softer than the normal cell. We also carried out supplemental studies by fluorescence microscopy and electron microscopy, which also supported our AFM results.

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Optical trapping of coated microspheres

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In an optical trap, micron-sized dielectric particles are held by a tightly focused laser beam. The optical force on the particle is composed of an attractive gradient force and a destabilizing scattering force. We hypothesized that using anti-reflection-coated microspheres would reduce scattering and lead to stronger trapping. We found that homogeneous silica and polystyrene microspheres had a sharp maximum trap stiffness at a diameter of around 800 nm - the trapping laser wavelength in water - and that a silica coating on a polystyrene microsphere was a substantial improvement for larger diameters. In addition, we noticed that homogeneous spheres of a correct size demonstrated anti-reflective properties. Our results quantitatively agreed with Mie scattering calculations and serve as a proof of principle. We used a DNA stretching experiment to confirm the large linear range in detection and force of the coated microspheres and performed a high-force motor protein assay. These measurements show that the surfaces of the coated microspheres are compatible with biophysical assays.

Micro & Nanotechnology: Nanopores

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Urea-Induced Conformational Changes in dsDNA Probed by Solid-State Nanopores

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Urea is an uncharged, polar molecule used ubiquitously as a structural denaturant for proteins, a hydrogen bond destabilizer in nucleic acids, and to increase the resolution of similar DNA fragments in electrophoretic processes. Despite its widespread use, the precise effects of varying urea concentrations on dsDNA structure are unknown. Solid-state nanopores in the range 2.7 - 4.5 nm have recently been shown to accurately gauge the effective diameter of dsDNA in aqueous solutions[1]. This DNA sizing method involves measurement of the reduction of a high ionic-strength electrolyte current as DNA transverses the pore. In this study, we systematically vary urea concentrations in the electrolyte solution (0 - 8.6M) and measure its effects on the effective DNA cross-section. Using an 800 bp dsDNA fragment, our studies reveal that the mean DNA effective diameter increases by as much as 0.6 nm. Further, the effective DNA diameter as a function of urea concentration follows a sigmoidal trend, indicative of cooperative binding. This interpretation is also supported by the translocation dynamics of the DNA as a function of urea concentration. We will present our experimental results along with a simple model to explain these observations.

[1]Wanunu, M., Sutin, J., McNally, B., Chow, A. & Meller, A. (2008) DNA Translocation Governed by Interactions with Solid State Nanopores. *Biophys J* 95, in press.

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Atomic Scale Description of Ionic Behavior in Polymer Nanopores

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Polyethylene terephthalate (PET) is a carbon-based polymer commonly used in plastic containers. Due to its high melting point, mechanical strength, and

selectivity for chemical modification, PET has become an excellent material for use in nanotechnology applications. In particular, PET membranes have been used to build very small pores with nanometer-scale diameters, so called "nanopores". Several interesting phenomena have been observed in PET nanopores, such as ionic current rectification, reverse rectification due to divalent cations, and nanoprecipitation. However, understanding the physical basis behind such phenomena is still a challenge. We have used molecular dynamics (MD) simulations to study the ionic transport properties of PET nanopores, including the conduction of KCl under different pH conditions and the effect of divalent ions on the ionic conduction and nanoprecipitation. To carry out these simulations, we have developed a protocol to build PET nanopores: First, we constructed a periodic model of bulk PET; then, we created a PET nanopore by removing atoms from a conical region and patching the exposed ends with benzoic groups, the PET surface reproducing the surface charge observed in experiments; finally, the PET nanopores are solvated and simulated under a variety of voltage biases using different ionic species, such as K(+1), Cl(-1), Ca(2+) and HPO₄(2-) ions. We applied the protocol and found that it resulted not only in good agreement with experimental data, but also provided an atomic description of the ion dynamics in PET nanopores. Specifically, we observed the enhancement of ionic current due to the surface charge, the permanent binding of Ca(2+) ions to the PET surface, and the dynamics of HPO₄(2-) ions inside PET nanopores.

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Nanopore Unzipping Of Ultra-long Dna Repeats For Single-molecule Mutation Detection

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Rolling-circle amplification (RCA) is an isothermal method for the hybridization-triggered enzymatic synthesis of hundreds to millions repeats of small, single-stranded, circular DNA. Using RCA, we create tandem repeats of a DNA sequence from human genome source, serving as a signal amplifier for ultra-sensitive detection of specific nucleic acids mismatches. Solid-state nanopores have been shown to be an extremely useful tool in probing and characterizing biopolymers on the single molecule level. In our recent study¹ sub-2 nm solid state pores have been successfully utilized to unzip small DNA duplexes, and detect base mismatches. Here we demonstrate for the first time that kilo-base RCA products can be characterized using solid-state nanopores, allowing us to enhance mismatch detection sensitivity and accuracy by hybridization with oligos² containing the consensus sequence. This study is an important milestone for the realization of single nucleotide polymorphism and for nanopore sequencing methodologies, demonstrating the feasibility of sequential unzipping, and translocation of extremely long ssDNA molecules.

1. McNally, B., Wanunu, M. & Meller, A. (2008) Electro-mechanical unzipping of individual DNA molecules using synthetic sub-2 nm pores. *Nano Letters* ASAP article 10.1021/nl802218f, in press.

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Salt Dependence Of RNA Translocations Through Solid State Nanopores

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Solid state nanopores have become a powerful tool to probe structural features of single biopolymers. Here, molecules are passed through a nanometer sized hole by a strong electrical field, causing a small change in the ionic current through the pore. Where previous studies have mostly focused on studying DNA, we present translocation results for single RNA molecules. In particular, we present current blockades of double-stranded RNA molecules at varying concentrations of background salt. Similar to what was found for DNA, our preliminary results suggest a crossover from current blockades at high salt (1M KCl) to current enhancements at low salt concentration (0.1 M KCl). This can be explained by an increasing contribution from the counter-ions screening the RNA backbone as the background salt concentration is decreased. These experiments demonstrate the strength of solid state nanopores in studying RNA, and pave the way towards unraveling more complex RNA structures through the use of solid state nanopores.

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Fabrication And Characterization Of Tunable, Low Stress Al₂O₃ Nanopores For The Electronic Detection Of Biomolecules

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Understanding the biophysics governing single molecule transport through solid state nanopores is of fundamental importance in working towards the goal of genome sequencing using nanopore based sensors. Here, we present a simple process for the fabrication and characterization of novel, low stress,

low noise aluminum oxide nanopores for biomolecule detection. Aluminum oxide has numerous attractive properties including high mechanical hardness, low surface charge, chemical inertness to strong acids and excellent dielectric properties from DC to GHz frequencies.

Device fabrication involved the use of Atomic Layer Deposition and Deep Reactive Ion Etching tools to form low stress, mechanically robust aluminum oxide membranes. High temperature process steps were avoided to allow for possible process integration with metal nano-electrodes and optical probes. The nanometer sized pores themselves were formed through Field Emission Gun Transmission Electron Microscope (FEG-TEM) based sputtering. We demonstrate the precise size tunability of these structures in the nanometer regime and examine the physics governing pore contraction in aluminum oxide. Diffraction patterns reveal polycrystallinity localized to the pore region post sputtering suggesting localized heating and possible thermal annealing under the electron beam. Film composition and thickness were characterized. In addition, we examine the surface charge properties of these structures as a function of buffer pH and molarity. The single molecule sensing ability of this novel structure was tested using dsDNA. Electrical characterization revealed a significant reduction in membrane capacitance and reduced high frequency dielectric noise relative to existing silicon nitride and silicon dioxide topologies. These improvements can greatly enhance device performance by improving sensitivity and signal-to-noise ratio. In summary, our work provides a novel yet simple approach to fabricate tunable, low stress chemically functionalizable nanopores for the detection of biomolecules.

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Asymmetric Spectral Characteristic of Ion Currents in Conical Nanopores

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The noise analysis of ion current signals through single nanopores is a critical problem in using nanoporous systems for biosensing. We have examined the noise characteristics of ion currents in single asymmetric polymer nanopores. These pores are conical in shape with openings of several nanometers at the cone tip. The pore walls are negatively charged at pH 8 due to the presence of carboxyl groups with density of one group per square nanometer. These conically shaped pores are cation selective and rectify the current with preferential direction for cation flow from the narrow entrance of the pore to the wide opening of the pore. With our electrode configuration, the average currents for negative voltages are higher than the average currents for positive voltages. We have found that the noise characteristics for the positive and negative currents are very different. The time signals were examined through power spectra analysis and Hurst analysis. At low salt concentration, the transient behavior of currents flowing in the direction from the narrow opening towards the wide opening show a power spectra with distinct 1/f behavior, where f is the frequency. The Hurst analysis of these currents reveals a deterministic component in the current behavior. In contrast, the spectra of currents from the wide opening towards the narrow opening show a thermal noise characteristic. We will discuss how these differences in the transient signals of ion current in conical pores reflect differences in the electrochemical potential of cations in the nanopores, and how they can be important for biosensing.

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Direct Probing of DNA/Nanopore Interactions Using Optical Tweezers

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Solid-state nanopores can be used to analyze the structure of long double-stranded DNA molecules and to probe their interactions with proteins. This method utilizes the native biopolymers' charge to electrically draw the molecules from the *cis* to the *trans* side of the pore. In small pores (<5 nm) the transport time of the biopolymer is determined by a balance of the electrical field and the frictional force resulting from interactions with the pore walls and hydrodynamic drag. Despite the central importance of biopolymer dynamics in virtually all nanopore applications, to date there have been no direct measurements of force in small pores during biopolymer transport. Here, we use optical tweezers to dynamically manipulate a λ -DNA molecule threaded through a <5nm pore while simultaneously recording force and ionic current. To characterize the interaction strength in the pore, we measure force/velocity profiles as a function of the applied voltage and ionic strength. By comparing experiments using different-sized pores, we quantify the relative contribution of interactions to the overall translocation dynamics. These measurements provide basic insight into the principles governing translocation in the interaction-dominated regime.

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Conductivity of Room Temperature Ionic Liquids in Single Nanopores

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