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New Developments in the Structural and Functional Investigation of Primary Cilia using AFM and Confocal Microscopy

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We have used Atomic Force Microscopy (AFM) in a force-measuring mode to map the specific locations of physiologically important channel proteins on the primary cilia of kidney epithelial cells. Primary cilia protrude from the apical membrane into the nephron cavity, function as a mechanochemical sensor, and are decorated with a rich variety of protein molecules. The distribution and role of channel proteins such as Polycystin-1 (PC-1) and Polycystin-2 (PC-2) are the subject of ongoing debate, related to Polycystic Kidney Disease (PKD) amongst others. Therefore we have adopted a multi-factorial microscopy approach to investigate the biophysical properties of the primary cilium. A combined AFM/confocal microscope has been used to compare the cilia (MDCK cell lines) AFM images, to those using immunofluorescence. Using imaging force spectroscopy we have correlated specific antibody-antigen recognition events with cilial topography, and observed the punctuate distribution of PC-1, PC-2 and β-Integrin, to nanometre resolution, an order of magnitude improvement on conventional immunofluorescence. We have also correlated AFM maps with elasticity and cilia persistence length. Finally we will demonstrate electrophysiological techniques to probe cilial mechanochemical sensor behaviour, correlated with AFM.

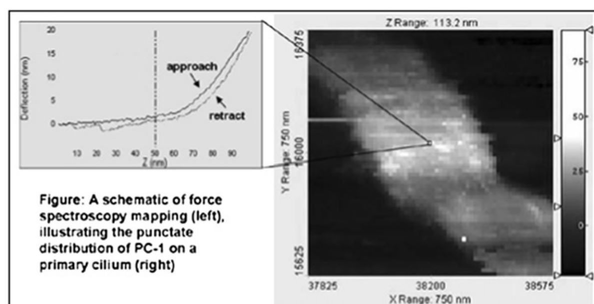

Figure: A schematic of force spectroscopy mapping (left), illustrating the punctate distribution of PC-1 on a primary cilium (right)

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Revealing Restriction Enzyme Dynamics With Fast-scan Afm

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Studying the mechanisms by which proteins interact with DNA is fundamental to understanding many biological processes. We studied two type II restriction enzymes: EcoRII and SfiI, which require binding to two or more recognition sites to cleave the DNA. We used a fast-scan atomic force microscope (fsAFM) with an average imaging rates of 2 frames/sec to image the dynamics of protein-DNA complexes, including the DNA cleavage reaction. We found different dynamics for both complexes. The EcoRII protein dissociates from the DNA synaptic complex leading to either a dimer or two monomers which stably interact with a single cognate site on the DNA. This protein was observed to slide over a range of about 300 base pairs to bind its second cognate site. The SfiI protein was seen to quickly dissociate with the formation of a detectable transient state. Also, evidence for an intersegmental transfer mechanism to bind a second site was observed for SfiI. The cleavage of the DNA by SfiI occurs in concerted manner similar to the model proposed earlier (Wentzell LM, et al., *J mol biol*, 1995).

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UVA Generates Pyrimidine Dimers In DNA Directly

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There is increasing evidence that UVA radiation, which makes up ~95% of the solar UV light reaching the Earth's surface, and also is commonly used for cosmetic purposes, is genotoxic. However, in contrast to UVC and UVB, the mechanisms by which UVA produces various DNA lesions are still unclear. In addition, the relative amounts of various types of UVA damages and their mutagenic significance are also a subject of debate. Here, we exploit atomic force microscopy imaging of individual DNA molecules, alone and in complexes with a suite of DNA repair enzymes and antibodies to directly quantify UVA damage and re-examine its basic mechanisms at a single molecule level. By combining the activity of endonuclease IV and T4 endonuclease V on highly purified and UVA-irradiated pUC18 plasmids we show, by direct AFM imaging that UVA produces a significant amount of abasic sites and pyrimidine dimers (CPD). However, we find that only about 60% of the T4 endonuclease V-sensitive sites, which are commonly counted as CPDs, are true CPDs, the other 40% being abasic sites. Most importantly, our results obtained by AFM imaging of highly purified native and synthetic DNA, using T4 Endonuclease V, photolyase and anti CPD antibodies, strongly suggest that CPD are produced by UVA directly. Thus, our observations contradict the predominant view that as yet unidentified photosensitizers are required to transfer the energy of UVA to DNA in order to produce CPDs. Our results may help to resolve the long-standing controversy about the origin of UVA produced CPD in DNA. This work is supported by the NSF and the NIH.

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AFM Visualization of the Interaction between MutS and Heteroduplex DNA

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Single base mismatches, which represent biosynthetic errors during DNA replication, are corrected by the mismatch repair system (MMR). One of the key players of the MMR reaction in E. coli is MutS, a protein that binds to the mismatch and initiates, with the help of other proteins, downstream events that involve the incision and partial degradation of the newly synthesized DNA strand. Details connecting mismatch recognition to downstream events are not fully understood at present. To gain better understanding of the modus operandi of MutS, we used atomic force microscopy (AFM) for direct visualization of MutS on heteroduplex DNA. Two different types of MutS/DNA complexes were captured by AFM. In the first type binding was observed at a single location on the DNA substrate and involved MutS dimers or tetramers. In the second type, DNA loops were identified, and at their bases, two closely associated MutS dimers were captured, possibly forming tetrameric complexes. Whenever a DNA loop was imaged, one of the MutS dimers was always found to be located on the mismatch. In the presence of ATP, one MutS dimer was still located on the mismatch, while the other MutS dimer was found to slide away from the mismatch site increasing the DNA loop size. Taken together these results suggest that loop formation and growth, which is orchestrated by two MutS dimers, one stationary, one mobile, may underlie the mechanism of search for the incision site. This work is supported by the NSF and the NIH.

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An Atomic Force Microscopy Study of the Mechanism of Cellulose Biodegradation

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Cellulose, a biopolymer consisting of long chains of β (1→4) linked glucose sugars, is used as structural material by plants, bacteria, and some animals, and is the most abundant polymer on earth. Degradation of cellulose to glucose, a sugar easily fermented to ethanol, occurs by the enzymatic hydrolysis of cellulose by cellulase enzymes. The enzymes have a complex structure including carbohydrate binding modules responsible for binding the enzyme to the cellulose and catalytic domains responsible for the biodegradation of cellulose. Atomic force microscopy (AFM) was used to study native cellulose films prepared from a bacterial cellulose source, Acetobacter xylinum, using a novel application of the Langmuir-Blodgett technique. These films allowed high resolution AFM images of single fibers and their microfibril structure to be obtained. Further in situ AFM imaging studies of single cellulose fibers were performed in solution using cellulolytic enzymes. By using genetically modified enzymes with either active or inactive catalytic domains, the catalytic and binding properties of the enzyme-cellulose system can be independently investigated. Studying the modular enzyme action separately will provide insight into the mechanism of cellulose binding and contribute to our understanding of the biodegradation process.