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Abstracts

– Single molecule biophysics –

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Helixlike pili is a prerequisite of uropathogenic *E. coli* to adhere to host and withstand urine flow

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Many infection processes start with primary adhesion of pathogenic bacteria to host cells. The Gram-negative uropathogenic *Escherichia coli* (UPEC) bacteria, invades the urinary tract region and cause in some cases severe infections, pyelonephritis, if they can withstand the rinsing action of urine and ascend to the kidney, via the bladder and ureters. To mediate adhesion, UPEC express quaternary surface organelles that are assembled from $\sim 10^3$ identical subunits into a helix-like coil, with a single adhesin located at the tip. It is believed that the single adhesin mediate attachment to host cells while the helix-like structures act as shock absorbers to dampen the irregularly shear forces induced by urine flow. To unravel the biomechanical properties of such quaternary structures, in particular in terms of their force-elongation and kinetic behavior, Force-Measuring Optical Tweezers (FMOT) have been used. A plethora of different types of pili have been identified in the literature and we show, using FMOT, that those dissimilarities might reflect the host environment. For example, we have found differences among pili expressed at diverse environment inside the urinary tract, which imply that pili presumably have evolved to resist specific forces under *in vivo* conditions. It is thus worth striving for understanding bacterial adhesion in order to figure out alternative to the over-abundance of antibiotics worldwide.

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Optical and electrophysiological detection of single phages across a lipid membrane

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We present an investigation study of the ejection of single T5 bacteriophages. In vivo studies of DNA ejections from the bacteriophage capsid show that the T5 genome is introduced in the bacterial host in two steps. First 8% of the genome is ejected then after a pause of a few minutes the rest is internalized. Bulk in vitro studies showed that various mutants of T5 eject their genome in solution following a single or a multistep process. By immobilizing single bacteriophages on a surface and following their ejection by fluorescence microscopy we showed that in all cases the ejection occurs in one step, but some mutants seem to have a subpopulation for which the triggering signal of the ejection is transmitted more slowly to the capsid entrance. We then reconstituted the phage receptor FhuA into giant liposomes and followed the ejection of the DNA into the liposome by fluorescence. Finally we incorporated FhuA in a suspended bilayer and followed the infection of the phages through the bilayer both by fluorescence labeling and electrophysiological measurements. We will discuss the influence of the cross membrane potential on the ejection speed of the DNA.

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Biofunctional micropatterned surfaces to study the spatio-temporal organisation of LFA-1

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Lymphocyte function associated antigen-1 (LFA-1) adhesion depends on receptor occupancy and lateral organization on the cell membrane. However, the signals and mechanisms which dynamically reorganize LFA-1 into high avidity clusters are still a subject of many studies. To obtain deeper insight on the mechanisms that control and regulate LFA-1 clustering, patterned surfaces of immobilized LFA-1 ligand areas were fabricated using microcontact printing.
The diffusion of LFA-1 expressed by monocytes stretched over patterned surfaces was followed in time using single molecule TIRF microscopy. Single LFA-1 nanocluster trajectories on individual cells showed an increase of immobile LFA-1 fraction and a slow-down of diffusing LFA-1 on the ligand areas compared to the non-ligand areas. Moreover, single-cluster intensity analysis indicated a reorganization of LFA-1 nanoclusters in microclusters upon ligand binding. Finally, single particle motion analysis of LFA-1 trajectories in close neighborhood to the ligand areas showed no assisted diffusion of LFA-1 towards the adhesive regions, consistent with random ligand-encountering and binding. We are currently investigating the effect of cell membrane organizers to regulate the spatio-temporal organization of LFA-1.
R. Diez-Ahedo et al, Small, in press.

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Direct observation of twisting steps during Rad51 polymerization on DNA

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The human recombinase hRad51 is a key protein for the maintenance of genome integrity and for cancer development. This protein plays a central role is the DNA strand exchange occurring during homologous recombination. Here we report the polymerization and depolymerization of hRad51 on duplex DNA observed with a new generation of magnetic tweezers, allowing the measurement of DNA twist with a resolution of 5° in real time. At odds with earlier claims, we show that, after initial deposition of a multimeric nucleus, nucleoprotein filament growth occurs by addition of single proteins, involving DNA twisting steps of 65±5°. Simple numerical simulations support that this mechanism is an efficient way to minimize nucleoprotein filament defects. This behavior, consisting of different stoichiometry for nucleation and growth phases, may be instrumental in vivo. Fast growth would permit efficient continuation of strand exchange by Rad51 alone while the limited nucleation would require additional proteins such as Rad52, thus keeping this initiation step under the strict control of regulatory pathways. Besides, our results combined with earlier structural information, suggest that DNA is somewhat less extended (4.5 versus 5.1 Å per bp) and more untwisted (18.2 versus 15 ° per bp) by hRad51 than by RecA, and confirm a stoichiometry of 3-4 bp per protein in the hRad51-dsDNA nucleoprotein filament.