creation by Gag remain controversial. Here we reveal that Gag adsorption and polymerization is modulated by membrane curvature. Negative curvatures stimulate formation of distinct fluid-like membrane domains tightly packed with Gag molecules, which further polymerizes into a stable protein shell. The nucleation of these domains happens at physiologically relevant high curvatures and Gag polymerization leads to stabilization of these highly bent membrane configurations. Our findings indicate a novel mechanism of negative curvature creation based upon curvature-driven polymerization of Gag and involving curvature-polymerization feedback.

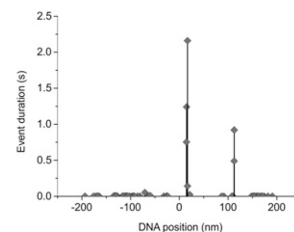
2140-Pos Board B159
Lentiviral Vectors Nano-Engineered with 'Marker of Self' CD47 to avoid Immune Surveillance
Nisha Sosale[1], Irena I. Ivanovska[1], Richard K. Tsai[1], Philip W. Zoltick[2], Yale Goldman[1], Dennis E. Discher[1].
[1]University of Pennsylvania, Philadelphia, PA, USA, [2]Harvard University, Boston, MA, USA.
Macrophages and dendritic cells take up foreign microbes from the circulation and other tissues and often present microbial components to the adaptive immune system. Uptake of viruses including Lentiviral Vectors (LVs) by macrophages not only makes viral delivery inefficient, but also contributes to an unwanted immune response to vector components as well as delivered gene products. Macrophage uptake of micron-size particles and cells is inhibited by CD47 display on the surface of target particles by an interaction with macrophage receptor SIRPa. A novel LV was engineered to present an oriented human CD47-GFP fusion protein on the vector envelope with the aim of reducing uptake by macrophages without affecting transduction of other cells – which is indeed demonstrated. While fluorescent microscopy, flow cytometry, and western blotting confirm that virus as well as the producing cells indeed express CD47-GFP, the key physical question is whether virus displays CD47-GFP in the proper orientation. AFM-coupled nano-fluorescence imaging of viral vectors displaying envelope CD47 demonstrated colocalization of the GFP tagged protein with acridine orange stained RNA, and AFM imaging also showed viral vectors were rigid and quasi-spherical, ranging in diameter from 100 to 300 nanometers as expected. Kinetics of lentiviral vector binding specifically to anti-CD47 coverslips established the proper orientation of CD47-GFP on the viral surface. This result confirms the development of a novel lentiviral vector that properly displays CD47 to specifically minimize macrophage uptake and subsequent immune activation to virus.

2141-Pos Board B160
Calcium-Mediated Fusion between Endo-Lysosomal Compartments Enhances Virus-Like Particles Release
Andrea Gramatica[1], Maik J. Lehmann[2], Roland Schwarzer[1], Andreas Herrmann[1].
[1]Humboldt University of Berlin, Department of Biology/Molecular Biophysics, Berlin, Germany, [2]Humboldt University of Berlin, Department of Biology/Molecular Parasitology, Berlin, Germany.
Assembly of the human immunodeficiency virus (HIV) is governed by the structural polyprotein Gag, which is necessary and sufficient for the release of virus-like particles (VLPs) from the host cell. Although the plasma membrane has been recognized as the major site for the production of VLPs, in some cell-types Gag is targeted to late endosomes/multivesicular bodies (LE/MVBs), where assembly and budding take place. Virus release into the extracellular space then occurs after regulated exocytosis. It is well accepted that the release of VLPs requires participation of different host cell components. In particular, it was shown that induction of a transient rise in cytoplasmic Ca^{2+} increased the amounts of VLPs in MVBs, and resulted in a dramatic enhancement of VLPs release (Perlman M. et al., 2006). However, although cellular factors have been already proposed as mediators of Ca^{2+} provision (Ehrlich L. et al., 2010), how Ca^{2+} can promote the release of VLPs remains to be determined. With FACS analyses on live cells, we could identify variations of intracellular Ca^{2+} in Gag-expressing cells treated with Ca^{2+} fluorescent indicators. High-resolution confocal and electron microscopy have confirmed that Gag can assemble and bud into VLPs in lysosomes (Ly) and LE. Furthermore, we could show for the first time that Ca^{2+} released specifically from those compartments causes formation of Ly/LE hybrid organelles, which in turn fuse with the PM and release VLPs into the extracellular space. This heterotypic fusion process requires components of the SNARE complex and the Ca^{2+} sensor protein Synaptotagmin VII, which regulates Ly exocytosis. All these elements constitute a productive pathway for virus assembly and release. We believe that the Gag protein itself, or a cellular factor recruited by Gag, might promote the increase of Ca^{2+} required for this process to function.

Protein-Nucleic Acid Interactions II

2142-Pos Board B161
Single Molecule Tracking of Lac Repressor Diffusing on Stretched DNA
Gionata Belcastro[1], Carina Mónico[1], Marco Capitanio[1,2], Francesco Vanzi[1,3], Francesco Saverio Pavone[1,2].
[1]LENS, Sesto Fiorentino, Italy, [2]Department of Physics, Sesto Fiorentino, Italy, [3]Department of evolutionary biology 'Leo Pardi', Florence, Italy.
Lac Repressor (LacI) is a DNA-binding protein that regulates genes expression by specific binding to its target sequence on DNA. This sequence is found rapidly among millions of base pairs in the genome, presumably via a combination of 3D and 1D-diffusion (following the interaction with non-specific DNA). Here we report diffusion analysis through the combination of single molecule localization and manipulation. For single molecule localization, Atto532 dye has been covalently linked to a single-cysteine mutant of LacI, LacIQ231C. A double optical tweezers system is used for trapping and stretching a single DNA molecule. All components of the experiment (protein, DNA, beads and buffer) are assembled (and/or rapidly exchanged) with a flow-system. This integrated configuration has several advantages: biomolecules are not in the proximity of the glass surface thus preventing possible electrostatic effects; controlled forces can be measured or applied. We characterized LacI 1D-diffusion under different forces applied to the DNA. The figure shows an example of the data obtained in the form of a kymogram.
Acknowledgments:The research leading to the results has received funding from Italian Ministry for Education, University and Research in the framework of the Flagship Project NANOMAX

2143-Pos Board B162
Lac Repressor-DNA Interactions assessed by Ultrafast Force-Clamp Spectroscopy
Carina Monico[1], Marco Capitanio[1,2], Gionata Belcastro[1], Francesco Vanzi[1,3], Francesco S. Pavone[1,2].
[1]LENS_ European Laboratory For Non Linear Spectroscopy, Sesto Fiorentino (FI), Italy, [2]Department of Physics and Astronomy, University of Florence, Sesto Fiorentino (FI), Italy, [3]Department of Evolutionary Biology, University of Florence, Florence, Italy.
We recently developed an ultrafast force-clamp laser trap technique [Capitanio *et al.*, Nature Methods 9,1013-1019(2012)] that allows probing, under controlled force, both long- and short-lived biomolecular interactions (100µs to tens/hundreds of seconds), as well as sub-nanometer conformational changes occurring upon bond formation. Here, we show the application of our method to the study of lactose repressor (LacI). Our results show two kinetically well-distinct populations of interactions, which clearly represent strong interactions (targeting the two operators located 100nm apart from each other: long events in the figure) and fast scanning of LacI along non-cognate DNA (during target-search: short events in the figure). Our results demonstrate the effectiveness of the method to study the sequence-dependent affinity of DNA-binding proteins along the DNA molecule and the effects of force on a wide range of interaction durations, including µs time scales not accessible to other methods. This improvement in time resolution provides also important means of investigation on the long-puzzled mechanism of target search on DNA and possible protein conformational changes occurring upon target recognition.



This research is funded by the Italian Ministry for Education, University and Research in the framework of the Flagship Project NANOMAX.

2144-Pos Board B163
Lactose Repressor Functions as a DNA Topological Barrier in *Escherichia Coli* Lactose Operon
Geraldine Fulcrand, Steven Eichelbaum, **Fenfei Leng**.
Florida International University, Miami, FL, USA.
Escherichia coli lac repressor (LacI) is a paradigm transcriptional factor that controls the expression of three genes in the *lac* operon. It is a tetrameric protein, specifically binds to *lac* operators, such as *O1*, *O2* and *O3*, and forms a DNA loop to negatively control transcription initiation. Previously, we found that LacI upon binding to multiple *lacO1* operators is capable of acting as a DNA topological barrier to block DNA supercoil diffusion and dividing a supercoiled DNA molecule into two independent topological domains (Leng et al. (2011) Proc Natl Acad Sci USA 108: 19973-78). In this study, we showed that LacI is able to function as a topological barrier and block supercoil diffusion upon binding