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.

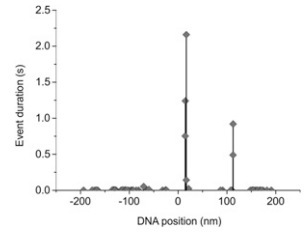
Acknowledgments: The research leading to the results has received funding from Italian Ministry for Education, University and Research in the framework of the Flagship Project NANOMAX

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

to O1, O2, and O3 operators in the *lac* operon. Specifically, we demonstrated that LacI kept two supercoils within the 401 bp DNA-loop between *O1* and *O2* operators. Additionally, We carried out time course studies to determine the stability of the topological barriers that are produced by the different LacI-operator complexes. Our results showed that the stability of the topological barriers correlates with the DNA-binding affinity of LacI to the different operators i.e., *O1*, *O2*, *O3*, and *Os* operators. Furthermore, we confirmed our previous observation in which LacI is able to ''keep'' certain superhelical energy to stabilize LacI-*lacO1* complexes. Our results can be explained by a model in which LacI behaves as a topological barrier in the *lac* operon to regulate the expression of *lacZYA* genes in *Escherichia coli* cells.

2145-Pos Board B164

Flanking DNA Matters in DNA Loop Formation and Breakdown Mediated by Lac Repressor Protein

Joel D. Revalee, Jens-Christian Meiners.
University of Michigan - Ann Arbor, Ann Arbor, MI, USA.

The lac Repressor protein (LacI) is a paradigm for the study of protein-mediated DNA loops in bacteria. When it binds to two distant operator sites on substrate DNA, it causes the formation of DNA loops. Although past in vitro and in vivo experiments have shown that changes in the inter-loop DNA can affect loop formation and breakdown, here I will present in vitro experimental evidence that the DNA outside the loop that is flanking the operators can also affect looping kinetics. Observing loop formation and breakdown in single molecules with Tethered Particle Motion (TPM) we found fluctuations in how much DNA is bound to the protein, and these fluctuations depend on the AT- or GC-content of the flanking regions outside the loop. This suggests an interaction between the looped complex and the flanking region which has not been previously observed. The studies complement previous experiments which found that DNA between the operator sites interacts with LacI while in the looped state. These results suggest that the presence or absence of flanking DNA interactions with the protein complex could give rise to additional states in TPM experiments that are not caused by conformational changes of the protein complex or topologic variations of the loop.

2146-Pos Board B165

DNA Competition Experiments Reveal the Importance of Operator Binding Strength and Inter-Operator Sequence in Protein-Mediated DNA Looping

Joel D. Revalee, Henry D. Wilson, Jens-Christian Meiners.
Univeristy of Michigan, Ann Arbor, MI, USA.

DNA looping mediated by the lac Repressor protein (LacI) is a paradigm of study for protein-DNA interaction as well as DNA mechanics on the nanoscale. Loop formation in this system occurs by the spontaneous binding of LacI to two distant operators on its DNA substrate. We employ single-molecule Tethered Particle Motion (TPM) to observe loop formation and breakdown in various DNA substrates. We are discussing and aiming to explain substantial differences between loop lifetime measurements in bulk and single-molecule experiments, namely an unexpected difficulty in competing bound protein off in the presence of excess DNA even though loops continued to form and break down rapidly. For this aim, we conduct single-molecule DNA competition experiments using DNAs with different operator strengths and intra-operator sequences giving rise to intrinsic bends. We find that we are able to compete off LacI that is bound to unbent DNA constructs with non-ideal operators in a matter of minutes, in line with typical loop breakdown rates. In contrast, competing off LacI bound to unbent or intrinsically curved DNA substrates with ideal operators took at least hours up to days in both single-molecule and bulk experiments, even though loops continue to break down and form repeatedly within minutes. To explain this resistance to competition of the LacI-DNA complex in DNA substrates with ideal operators while loops continue to break down, we posit a weak binding of LacI to non-operator DNA in the unlooped state; this hypothesis is supported by recent complementary experiments that reveal interactions between LacI and non-operator DNA outside the looping region.

2147-Pos Board B166

Analysis of DNA Looping Kinetics in Tethered Particle Motion Experiments using Hidden Markov Models

Martin Lindén[1,2], Stephanie Johnson[2,3], Jan-Willem van de Meent[4], Rob Phillips[2], Chris H. Wiggins[4].
[1]Stockholm University, Stockholm, Sweden, [2]California Institute of Technology, Pasadena, CA, USA, [3]University of California, San Fransisco, CA, USA, [4]Columbia University, New York, NY, USA.

Tethered particle motion (TPM) is a powerful method for measuring DNA-protein interactions at the single molecule level. TPM experiments monitor the Brownian motion of beads tethered to a microscope cover slip. The Brownian motion changes when a protein binds to and deforms the DNA 'leash', for example in the formation of a DNA loop. A complicating factor in the interpretation of TPM data is that the number of observable states, corresponding to different conformations of the DNA-protein complex, is often not known in advance. Moreover, conformational transitions that occur on time scales comparable to the diffusive motion of the bead are difficult to extract from the data. We present an analysis method for TPM data that overcomes these limitations in existing approaches. Our method relies on variational Bayesian inference on a variant of the Hidden Markov model. This variational approach allows us to determine the number of states directly from the data in a statistically principled manner.
Moreover, by operating directly on the position data, we achieve significantly better time resolution compared to methods based on running averages of the bead root-mean-square distance from the tethering point. Finally, we show that hierarchical techniques developed in the context of single molecule FRET experiments can be adapted to our TPM methods to perform pooled analysis on many trajectories. This increases the accuracy of the method despite considerable bead-to-bead variability, and allows a more precise characterization of rare events.
We apply our method to Lac-mediated loop formation on a short (107 bp) construct, and demonstrate direct interconversion between two different looped states, with implications for structual models of the looped Lac-DNA complex.

2148-Pos Board B167

Binding to Different Homologous DNA Sequences by a Protein alters the Dynamical Properties of the Bound Protein in a DNA Sequence Dependent Manner: Operator DNA Induced Allostery in the Structure and Dynamics of Lambda Repressor

Tanumoy Mondol, Samir Kumar Pal.
SNBNCBS, Kolkata, India.

Δ-repressor-operator sites interaction, particularly O_R1 and O_R2, is a key component of the Δ-genetic switch. The N-terminal domain of the protein Δ-repressor interacts with the DNA, while the C-terminal domain is responsible for most of the protein–protein interactions, essential for the co-operative binding and for the functioning of the genetic switch. Frster resonance energy transfer from the dansyl, bound to the C-terminal domain of the protein, to the intercalated EtBr in the operator DNA, indicates that the structure of the protein is more compact in the O_R2 complex than in the O_R1 complex. We have explored the photoinduced electron transfer process from the tryptophan moieties of Δ-repressor to O_R1 and O_R2 DNA to verify the conformational differences of the C-terminal domain of the repressor, bound to O_R1 and O_R2 DNA. Most importantly, fluorescence anisotropy study reveals enhanced flexibility of the C-terminal domain of the repressor at ultrafast timescales upon complexation with O_R1. In contrast, O_R2 bound repressor shows no significant enhancement of protein dynamics at these timescales. Moreover, sedimentation equilibrium study reveals that this differential dynamics is important for correct protein-protein interactions between two Δ-repressor dimers bound to O_R1 and O_R2, for the functioning of the genetic switch. Hence, we demonstrate that binding of transcription factors to specific DNA sequences alters the dynamical properties of the bound protein in a DNA-sequence dependent manner and the dynamical difference contributes to the formation of correct regulatory complex.
References:
1. *Mondol, T. et al. FEBS Lett. 586 (2012) 258 (Cover Article).*
2. *Mondol, T. et al.J. Biomol. Struct. Dyn. 30 (2012) 362.*

2149-Pos Board B168

The Different FMRP Isoforms Bind with High Affinity to the G-Quadruplex formed by the FMRP mRNA

Anna Blice-Baum, Rita Mihailescu.
Duquesne University, Pittsburgh, PA, USA.

Fragile X syndrome, the most common form of inherited mental retardation in humans, affects about 1 in 3000 males and 1 in 5000 females. It is caused by the loss of expression of the fragile X mental retardation protein (FMRP) due to a CGG trinucleotide repeat expansion in the 5'-untranslated region (UTR) of the fragile x mental retardation-1 (fmr1) gene. FMRP has been shown to use its arginine-glycine-glycine (RGG) box RNA binding domain to bind with high affinity and specificity to G quadruplex forming mRNA sequences. The binding of FMRP to a proposed G quadruplex structure in the coding region of its own mRNA (100 nucleotide fragment named FBS) has been proposed to affect mRNA splicing events for isoforms 1 through 3. In this study we truncated the original 100 nt FMRP-FBS to 42 nt and used biophysical methods to directly demonstrate its folding into a G-quadruplex structure and the binding affinity of the different FMRP isoforms to it.