that Klenow and Klentaq have minimal sensitivity to pH changes, with proton linkages of ~0.06 and ~0.3 respectively in KCl. Furthermore, osmotic stress data in KCl indicates 500~600 waters are released upon binding by both polymerases.
Glutamate is the major intracellular anion accumulated in *E.coli* in the presence of KCl in the external environment. The 'glutamate effect' is primarily characterized by an increase in DNA binding affinity when chloride is replaced by glutamate. Some proteins also exhibit decreased ionic linkage in glutamate. Klenow exhibits both aspects of the 'glutamate effect'. Substituting glutamate for chloride reduces the ionic linkage for Klenow by >50%. The presence of glutamate also increases the proton linkage of Klenow five fold and decreases water release by ~70% to approximately 150 waters. The dramatic decrease in water release highlights the osmotic nature of the glutamate effect. Glutamate and chloride salts behave as ionic inhibitors of DNA binding but glutamate salts also exhibit an osmotic enhancement effect.
While Klentaq's DNA binding affinity is also enhanced by glutamate, its ionic and proton linkages are not altered. The osmotic enhancement is present for Klentaq but it is not as significant in the salt concentration range at which nanomolar Klentaq-DNA binding occurs. *E.coli* DNA-binding proteins might have evolved to bind tightly at higher salt concentration to utilize the glutamate effect while accumulating intracellular glutamate.

2158-Pos Board B128
Atomic Force Microscopy In Solution Shows Nucleosome Positioning By Excluding Genomic Energy Barriers
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Because of the importance of the nucleosomal organization in processes such as replication, transcription, DNA repair and recombination, understanding the role of DNA sequence on the chromatin organization is one of the main challenges in functional genomics. In this context, we have studied the positioning of reconstituted nucleosomes on genomic 400-900bp Saccharomyces cerevisiae and human DNA fragments, including two promoter regions, by coupling AFM imaging in liquid with a physical modeling of nucleosome formation energy based on sequence-dependent DNA bending properties. An important result coming out from these studies is the excluding role of high energy barriers that prevent nucleosome formation (nucleosome free regions) that contributes to the global nucleosome organization of the chromatin fiber by some ''parking phenomenon''. The investigation of a yeast and a human gene promoter regions, that are respectively positively and negatively regulated by a chromatin organization, confirms the existence of energy barriers as well as their major impact on the positioning of these nucleosomes.
Altogether, these results show that nucleosome positioning by genomic energy barriers has a main role in the nucleosomal array assembly and is likely to be a key to the understanding of chromatin mediated regulation processes.

2159-Pos Board B129
Rapid Formation And Breakdown Of Protein-mediated DNA Loops
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The Lactose Repressor protein (LacI) is a paradigm for the study of transcriptional regulation and protein-DNA interaction. LacI represses transcription of the Lac operon in E. Coli by binding to two distant operator sites and bending the intervening DNA into a DNA loop. Despite a wealth of knowledge on the biochemistry of this process, the details of the binding dynamics are still unresolved, and are the subjects of several lines of current investigations. We present Tethered Particle Microscopy (TPM) data on designed hyperstable loop-forming DNA constructs and find that LacI-mediated DNA loops form and break down on time scales of the order of minutes. This is in stark constrast to measurements in competition assays by Mehta et al[1], who report loop lifetimes of days for these constructs. We propose two possible explanations for the LacI-loop formation process that hamronizes these seemingly contradictory observations. Specifically, we propose that the loop-forming LacI tetramer is destabilized by binding to the DNA, and that therefore the primary loop breakdown process is a dissociation of the tetramer into two DNA-bound dimers, which is in contradiction to the prevailing model for this process. Alternatively, we discuss what assumptions have to be made to explain these experimental results purely in terms of dissociation of the tetramer from DNA. Namely, we need to assume an outsized effect of spatial operator orientation on loop formation rates and postulate that the protein is extremely inflexible.

1. Mehta, R.A. and Kahn, J.D. ''Designed hyperstable lac-Repressor-DNA loop topologies suggest alternative looop geometries.'' Journal of Molecular Biology 294 (1999), 67-77.

Virus Structure & Assembly

2160-Pos Board B130
Retrovirus and the Cytoskeleton: Insights Into the Mechanism for Viral Assembly
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The assembly and budding of a new virus is a fundamental step in retroviral replication. Yet, despite substantial progress in the structural and biochemical characterization of retroviral budding, the underlying physical mechanism remains poorly understood. In particular, the mechanism by which the virus overcomes the energy barrier associated with the formation of high membrane curvature during viral budding is unknown. Using atomic force- and transmission electron microscopy we find that both human immunodeficiency virus and Moloney murine leukemia virus remodel the actin cytoskeleton of their host cells and utilize the forces it generates to drive their assembly and budding. Highly dynamic actin-filamentous structures which varied in size over the duration of budding appeared to emanate from the assembled virion. These actin structures assemble simultaneously or immediately after the beginning of budding, and disappear as soon as the nascent virus is released from the cell membrane. Analysis of sections of cryo-preserved virus infected cells by transmission electron microscopy reveals similar actin filaments structures emerging from every nascent virus. Substitution of the nucleocapsid domain implicated in actin binding by a leucine-zipper domain resulted in budding of virus-like particles that was not accompanied by remodeling of the cell's cytoskeleton. Notably, budding of viruses carrying the modified nucleocapsid domains was an order of magnitude slower than that of the wild type. The results of this study show that retroviruses utilize the cell cytoskeleton to expedite their assembly and budding.

2161-Pos Board B131
Capsid Assembly in Small, Unenveloped Icosahedral DNA and RNA Viruses
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There are two distinct mechanisms for the assembly of viral capsids. (1). In some cases, such as DNA bacteriophage, protein-protein interactions are strong, and protein-nucleic acid interactions are weak. Under suitable conditions, the proteins assemble into a capsid spontaneously, and the nucleic acid is then driven into the capsid by an ATP-driven motor. (2) In other cases, such as some single-stranded RNA viruses, protein-protein interactions are weak, while protein-nucleic acid interactions are strong. The capsids of these viruses do not assemble in the absence of the genome, but viral assembly occurs spontaneously if both the nucleic acid and the protein are present; no energy source is required. We are investigating both of these processes, using coarse-grained molecular mechanics models. The simplest model treats the assembly of bacteriophage capsids using a truncated triangular pyramid to represent the asymmetric unit, with van der Waals forces to promote association. For this model, the size and structure of the aggregates is sensitive to the dihedral angle between subunits (i.e., to the angle at the peak of the pyramid). Over a small range of angles, a solution containing isolated subunits at submicromolar concentrations will quickly assemble into spherical aggregates with twenty subunits each, and these become icosahedral when the temperature is lowered; this corresponds to the formation of T=1 viral particles. We are exploring a number of issues for these model bacteriophage, including: conditions yielding T=3 and higher structures; assembly of asymmetric units and mature viruses from quasi-equivalent monomers; and the effects of model cations.

2162-Pos Board B132
Assembly of Viruses and the Pseudo Law of Mass Action
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The self-assembly of viral capsids is believed to obey the Law of Mass Action (LMA), this despite the fact that viral assembly is not a reversible process. We present a soluble model for irreversible capsid assembly, the ''Assembly-Line Model'' (ALM) and show that, in this model, viral assembly from a supersaturated solution of capsid proteins is characterized by a shock front that propagates in the assembly configuration space from small to large aggregate sizes. If this shock front is able to reach the size of an assembled capsid, then a transient state develops characterized by a ''pseudo'' LMA that would be difficult

to distinguish from a state of true thermodynamic equilibrium. The pseudo LMA describes partitioning of capsid proteins between assembled capsids and a metastable, supersaturated solution of free proteins. This metastable state decays logarithmically slowly. We show that the line energy of assembly intermediates is the key control parameter of the pseudo LMA.

2163-Pos Board B133
Mechanisms Of Viral Capsid Assembly Around A Polymer
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We present a coarse-grained computational model inspired by the assembly of viral capsid proteins around nucleic acids or other polymers. Specifically, we simulate on a lattice the dynamical assembly of closed, hollow shells composed of several hundred to 1000 subunits, around a flexible polymer. As a function of capsid size, we determine the maximum polymer length that can be dynamically encapsidated and the polymer length around which assembly is most effective. The assembly process can often be described by three phases: nucleation, growth, and a completion phase in which any remaining polymer segments are captured. We find that the polymer can increase the rate of capsid growth by stabilizing the addition of new subunits and by enhancing the incoming flux of subunits. We determine the relative importance of these mechanisms as a function of parameter values, and make predictions for the dependencies of assembly rates and effectiveness on polymer length. These predictions can be tested with bulk experiments in which capsid proteins assemble around nucleic acids or other polymers; in addition, we will discuss how predictions for the polymer-length dependence of assembly rates during the growth phase can be tested with single molecule experiments.

2164-Pos Board B134
Conformational Changes Of Gag HIV-1 On A Tethered Bilayer Measured By Neutron Reflectivity Provides Insights Into Viral Particle Assembly
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Formation of the HIV-1 is mediated by the Gag polyprotein at the cytoplasmic membrane surface of the infected host cell. Individual Gag molecules contain several domains connected by flexible linkers. Early cryo-EM data showed Gag in the immature virus as elongated rods radial from the membrane with one termini tightly bound to the viral genome [Current Biology, 1997 (7) p. 729]. However, solution measurements using SANS and other techniques suggest a compact structure for Gag [J. Mol. Biol. 2007 (365) p. 812]. These studies indicate large conformational changes in the Gag protein must occur concomitant with virus assembly. The dimension of Gag bound to the bilayer interface was determined at high resolution by neutron reflectometry. The bio-mimetic environment for observing Gag association consisted of a supported membrane attached to a gold surface via a PEO tether. The membrane was a ternary composition of DMPS:DMPC:Cholesterol lipids capturing key characteristics of the viral lipodome. First, the orientation of the membrane binding Matrix domain of the Gag protein was modeled using high resolution X-ray structures. Then measurements using the full length Gag protein bound to the lipid membrane showed Gag adopting a folded conformation. Upon addition of a 14 base pair DNA oligo (TGx7), a significantly thicker protein layer of ~200 Å was observed. A high salt buffer rinse reversed the conformational change. These results suggest a mechanism by which Gag extension is possible only once bound to the plasma membrane and in the presence of the viral genome. This provides a picture consistent with earlier in vivo and solution studies. A detailed understanding of the viral particle assembly process may elucidate susceptible points providing opportunities to inhibit proper virus formation.

2165-Pos Board B135
Visualizing The Biogenesis Of Individual Hiv-1 Virions In Live Cells
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The genesis of individual virus particles has never been observed in real-time. Consequently, some basic properties of virus particle assembly, such as kinetics and location, are unknown. Using several techniques based on total internal reflection fluorescent microscopy (TIR-FM), and live cells expressing fluorescent protein-tagged derivatives of Gag, the major structural component of HIV-1, we were able to observe and quantitatively describe the genesis of hundreds of individual virions in real time, from initiation of assembly to budding and release.
In Hela cells expressing a mixture of Gag-GFP and untagged Gag, a bright and diffuse fluorescent signal in the TIR field was detectable a few hours after transfection. One to two hours later, Gag puncta started to appear, at the rate of few virions per minute. Individual puncta appeared slowly, over minutes, and remained static during this period and thereafter. FRET and FRAP analysis demonstrated that the emergence of these appearing puncta was accompanied by a recruitment of Gag molecules that become progressively more proximal to each other until they segregate from the cytoplasmic pool. By fusing Gag to a GFP variant that is not fluorescent at acidic pH and by varying the cytoplasm pH with a pulse of pCO2, we showed that the fluorescence of a population of virions exhibited low sensitivity to pH changes. These virions were therefore not attached to the cell anymore and had completed assembly by budding.
Our analysis shows that HIV-1 particle genesis is initiated and completed at the plasma membrane and that a typical HIV-1 particle requires five to six minutes to complete assembly. Overall, these approaches have allowed an unprecedented view of the genesis of individual virus particles. We are currently investigating the recruitment of cellular components to nascent virions.

2166-Pos Board B136
The Nature Of Influenza Virus Virulence/Pathogenicity
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Based on pathogenicity influenza viruses can be classified as highly pathogenic (HP) or low pathogenic (LP). We analyzed genomes of HP H5N1 viruses isolated from chickens in Nigeria (Owoade, 2008) and found unique mutation in haemagglutinin, which may affect structure of antigenic region of HA and, therefore, may allow the virus to escape from the host immune responses. We also analysed genomes of LP viruses isolated from wild birds in Nigeria and found mutation at in the non-structural protein NS1. This mutation may destabilize NS1 interaction with the cellular CPSF30 protein which is normally occurs during HP virus infection (Das, 2008) and, therefore, may induce the antiviral responses. We will also disscuss distinct cellular processes which the HP and LP viruses relay on or suppress.

2167-Pos Board B137
Analysis Of Influenza Hemagglutinin Ligand-binding From Mutational Data And Molecular Motion
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Influenza hemagglutinin binds to sialic-acid-terminated glycans on the surface of target cells. This ligand-binding specificity of hemagglutinin is believed an important determinant of which host species it infects. Site-directed mutagenesis of hemagglutinin, expression, and determination of ligand-binding affinity are feasible but technically involved, so only a few hemagglutinin mutations have been tested in this manner. To understand the mechanism of binding specificity and guide further experiments, we have analyzed H5N1 avian influenza isolates to predict residues important to ligand binding and ultimately to ligand specificity. We employed sequence data from all available isolates in combination with analysis of protein residues that correlate with ligand conformation in molecular dynamics simulations to generate candidate sites for mutation. Using this combined analysis, we have predicted five residues both in the sialic-acid-binding site of hemagglutinin and more distant from it. In an initial evaluation, we have performed extensive molecular dynamics simulation of twelve point mutations at these sites. We simulated each of the 12 mutants in 3x100 ns and 200x10 ns simulations to obtain more robust statistical estimates of ligand dissociation. These simulations indicate a greatly increased dissociation rate from the mutants compared to simulations of wild-type H5N1 hemagglutinin VN1194, indicating that the mutations may disrupt ligand binding as expected. This analytic technique may thus provide an important means of screening potential binding-specificity mutants of influenza hemagglutinin as well as a more general tool to assess residues involved in ligand binding.

2168-Pos Board B138
High resolution optical microscopy analysis of Influenza Virus A assembly
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Novel, advanced imaging tools, like the subdiffraction-limit fluorescence microscopy techniques STORM/PALM (stochastic optical reconstruction microscopy/photoactivation localization microscopy) are exquisitely suited to illuminate and dissect the high complexity and molecular mechanisms of biological processes at nanoscopic scale. These techniques make use of activating only a subset of fluorescent molecules at a time, which allows determining their localization with nanometer precision. Here, we are applying these tools to study the intricate relationship of host-virus interactions at single-particle level, using Influenza virus A as model system.