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