shorter lengths. For all of these modifications, our results show no systematic change in the abortive amounts or profile.
A much simpler model argues that short RNA:DNA hybrids are intrinsically unstable simply because of their short lengths. In both the T7 and E. coli systems, we show that stabilizing the hybrid by initiating transcription using a pyrene-conjugated RNA dinucleotide eliminates abortive cycling altogether (the pyrene is known to add stabilizing stacking interactions). This result further argues against the scrunched intermediate model in that addition of the extra pyrene bulk should increase steric stress and so increase abortive cycling.

366-Pos
An Abortive Isomerization Branch in the Transcription Initiation Pathway At a σ^{54} Promoter As Revealed By Single Molecule Fluorescence Microscopy
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Regulated transcription initiation is a complex process that involves multiple protein factors and a series of polymerase-DNA complexes that are intermediates in the reaction. This complexity presents a significant challenge for ensemble experiments that aim to elucidate the reaction pathway. We here report the kinetic mechanism of initiation at the σ^{54} promoter of the glnALG operon in *Salmonella typhimurium*. This prototypical activator-dependent promoter is regulated by nitrogen stress. To circumvent the complexity of ensemble analysis, we used multi-wavelength single-molecule fluorescence colocalization methods to follow initiation reactions on individual surface-anchored DNA molecules that contain σ^{54} promoters. Three distinguishable dye labels enabled us to follow reactions in which RNA polymerase binding, open complex formation, escape into transcription elongation and departure of the σ^{54} subunit were detected in individual transcription complexes, and the interconversion kinetics for all states were measured. Transcription initiation from this promoter occurs only following a polymerase isomerization that is induced by interaction with the NtrC activator protein in the presence of ATP. However, we observed that with NtrC present the polymerase leaves the promoter faster than the combined rates of initiation plus closed complex departure. Thus, a fraction of activator-mediated polymerase isomerizations displace the polymerase from the promoter without initiating transcript synthesis. This activator-induced abortive isomerization is a non-productive branch off of the initiation pathway and is more common than productive transcription initiation. We speculate that abortive isomerization is a consequence of the large energy input required to disrupt promoter-polymerase interactions prior to promoter escape. Taken together, our results define the full pathway and dynamics of initiation at this activator-dependent promoter and illustrate the power of multi-wavelength single-molecule colocalization methods in the elucidation of complex biological regulatory mechanisms.

367-Pos
Highly Bent DNA: A Novel Repressor of T7 RNA Polymerase
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The use of DNA templates sustaining varying degrees of supercoiling has established that mechanically stressed DNA can influence transcription by RNA polymerase (RNAP). However, the interpretation of supercoiling studies is complicated by the lack of a detailed description of the bending and torsional conditions present on length scales that are relevant to RNAP activity. A quantitative understanding of how bending and twisting DNA influence transcription has yet to emerge, largely owing to the lack of an assay capable of quantifying the transcriptional competency of an RNAP from DNA templates sustaining well-defined levels of mechanical stress in the absence of supercoiling or other DNA-binding proteins. To directly test the hypothesis that mechanical stress imparted to tightly looped DNA is sufficient to repress transcription, we have developed an assay capable of quantifying the ability of bacteriophage T7-RNAP to transcribe circular transcription templates on the order of 100bp in size, thus restricting our observations only to the effects of mechanical stress on transcription. By encoding the promoter sequence for T7-RNAP within minicircles 100bp, 106bp, and 108bp in size, we have also characterized the effects of three distinct torsional stress states (within comparably bent minicircles) on the transcriptional activity of T7-RNAP. From these minicircle templates, we observe that the elongation velocity and processivity of T7-RNAP is reduced by roughly two orders of magnitude, confirming that highly bent DNA alone is capable of repressing transcription. Additionally, we observe a fivefold enhancement of elongation velocity as the template is untwisted, a finding qualitatively supported by previously reported observations. Our results establish that DNA mechanics can directly control RNAP activity, and given the required use of DNA templates by all RNAPs, necessitate the consideration of template-mediated effects in repression studies.

368-Pos
Single Molecule Study of Promoter Search By E Coli RNAP
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During transcription initiation, RNA polymerase (RNAP) must find specific promoters in the genome in response to different physiological conditions. It has been suggested that 1-D sliding along DNA may accelerate this process so that it can be faster than 3-D diffusion limit. However contradicting ensemble and single molecule experiments have reported drastically different 1-D diffusion coefficients ($10^{-1}\mu m^2 s^{-1}$ vs. 10^{-2} and 10^{-5} $\mu m^2 s^{-1}$). Here we used our high throughput single molecule technology to simultaneously observe hundreds of double tethered lambda DNA molecules in an effort to determine how Qdot-labeled *E. coli* RNAP searches for promoters. Using this system we have observed specific binding to known promoters, formation of heparin resistant open complexes, and transcription from know promoter regions. Analysis of the time courses of promoter search showed two populations: The first population binds DNA nonspecifically and dissociates with an average life time 3.5 sec; The second population binds DNA specifically to the promoter regions and never comes off within our observation time. We have not observed evidence of extensive 1D diffusion with either population, and we estimate upper boundaries for the diffusion coefficients and sliding lengths of $10^{-4}\mu m^2 s^{-1}$ and 170bp, respectively; these values are much smaller than reported by ensemble experiments. Our data suggest that 3-D diffusion is the main pathway for *E. coli* RNAP to search for promoters and 1-D sliding does not play a significant role in this process. The biological context of this result is discussed.

369-Pos
Structural Modeling of PhoB Dimer and Its Interaction With RNAP Complex
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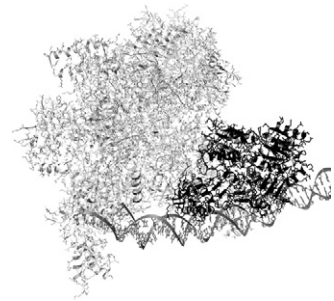
PhoB is a response regulator of the two-component signal transduction system. Structurally, PhoB can be divided into two domains. A N-terminal Receiver Domain (ED) that adopts a flavodoxin-like fold shared by receiver domains of other response regulators. The C-terminal Effector Domain (ED) of PhoB adopts a winged-helices fold that recognizes and binds to its targeted DNA duplex. Structures of PhoB molecule have been well-studied including the homodimers of the ED (PDB accession code: 1GXP), the RD (PDB accession code: 2JB9) and the two domains structure (PDB accession code: 1KGS). However, the functional form (DNA-binding) of the PhoB two-domains structure is still not available. Here, we engaged in an exercise to develop a structural model of the molecule in its dimeric functional form binding to its targeted DNA duplex. The model was developed using the observed crystal contacts between the domains of various response regulators. The modeled structure of PhoB/DNA complex is assembled into the RNAP/DNA complex (also modeled by our group) to study the interactions between PhoB and RNAP as shown in the attached figure.



370-Pos
Quantitative Studies of Transcription in E.coli With Subdiffraction Fluorescence Microscopy
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The organization of biomolecules into macromolecular assemblies is often closely related to biomolecular function. However, such structures often remain unresolved using conventional light microscopy. By applying novel high-resolution single-molecule fluorescence techniques, it becomes possible to study biomolecular structure and interaction below the diffraction limit of light, reaching a lateral resolution of ~20 nm [1, 2]. We use photoswitchable and photoactivatable fluorescent probes in combination with direct stochastic optical reconstruction microscopy (dSTORM) [2] and photoactivation-localization microscopy (PALM) [3]. Following light-induced activation of a subset of fluorescent probes attached to target proteins, the fluorescent state is read out and single emitters are localized with nanometer precision. This procedure is

repeated many times, and the ensemble of coordinates is used to reconstruct an image with superior resolution [2, 3].
RNA polymerases have been localized in bacteria using conventional approaches [4]. In our study, we focus on the spatial organization of bacterial transcription sites in E. coli at the molecular scale. To reach that goal, we apply high-resolution fluorescence methods, and we will present a refined understanding of structure and function in the bacterial transcription machinery.
References:
[1] van de Linde et al. (2009) *Multicolor Photoswitching Microscopy for Subdiffraction Resolution Fluorescence Imaging*, Photochemical and Photobiological Sciences, 8, 465-469.
[2] Heilemann et al. (2008) *Subdiffraction-Resolution Fluorescence Imaging with Conventional Fluorescent Probes*, Angew. Chemie, 47, 6172-6176.
[3] Betzig et al. (2006) *Imaging Intracellular Fluorescent Proteins at Nanometer Resolution*, Science, 313, 1642-1645.
[4] Jin, D.J.; Cabrera, J.E. (2006) *Coupling the Distribution of RNA Polymerase to Global Gene Regulation and the Dynamic Structure of the Bacterial Nucleoid in Escherichia coli*, Journal Structural Biology, 156, 284-291.

371-Pos
A Microfluidics-Based Platform For Identification and Detailed Characterization of Transcription Factor Binding Sites
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Transcriptional regulation of gene expression is mediated by transcription factors that bind DNA sequence-specifically within gene promoters to activate or repress transcription. However, biochemical data linking transcription factors to their consensus binding sites has traditionally been difficult to obtain, complicating reconstruction of cellular pathways contributing to a transcriptional response.
We have developed a versatile and sensitive microfluidics-based technique for *de novo* identification and subsequent detailed characterization of transcription factor consensus motifs and binding energy landscapes. Our technique offers several advantages over current methods. First, our technique mechanically traps all complexes at equilibrium prior to measurement, allowing detection of weak or transient interactions and providing direct, quantitative measurements of reaction parameters. Second, our technique requires extremely small amounts of reagents, permitting protein production via cell-free transcription/translation of PCR-generated templates and eliminating laborious and time-consuming cloning steps. Finally, our technique allows high-throughput screening of transcription factor binding to all possible DNA 8mers in a single experiment.
To evaluate the performance of our new technique, we probed DNA binding patterns for 30 yeast transcription factors from various families and used a statistical-mechanical model of transcription factor binding to determine preferred consensus motifs. In all cases, the core consensus obtained agreed with previous literature results, validating the utility of our technique for *de novo* identification of transcription factor binding sites. This quantitative data set provides critical information that can be used to revise and refine current models of transcription factor binding interactions.

372-Pos
Model of Transcriptional Activation By MarA in Escherichia Coli
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The AraC family transcription factor MarA activates ~40 genes (the *marA/soxS/rob* regulon) of the *Escherichia coli* chromosome resulting in different levels of resistance to a wide array of antibiotics and to superoxides. Activation of *marA/soxS/rob* regulon promoters occurs in a well-defined order with respect to the level of MarA; however, the order of activation does not parallel the strength of MarA binding to promoter sequences. To understand this lack of correspondence, we developed a computational model of transcriptional activation in which a transcription factor either increases or decreases RNA polymerase binding, and either accelerates or retards post-binding events associated with transcription initiation. We used the model to analyze data characterizing MarA regulation of promoter activity. The model clearly explains the lack of correspondence between the order of activation and the MarA-DNA affinity, and indicates that the order of activation can only be predicted using information about the strength of the full MarA-polymerase-DNA interaction. The analysis further suggests that MarA can activate without increasing polymerase binding and that activation can even involve a *decrease* in polymerase binding, which is opposite to the textbook model of activation by recruitment. These findings are consistent with published chromatin immunoprecipitation assays of interactions between polymerase and the *E. coli* chromosome. We find that activation involving decreased polymerase binding yields lower latency in gene regulation and therefore might confer a competitive advantage to cells. Our model yields insight into requirements for predicting the order of activation of a regulon and enables us to suggest that activation might involve a decrease in polymerase binding, which we expect to be an important theme of gene regulation in *E. coli* and beyond.

373-Pos
Transcription Factor Switching Dynamics Regulates Gene Activation
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Molecular mechanisms controlling the time diffusing molecules take to activate specific target proteins are pivotal for cellular response and signaling. We study the activation dynamics when diffusing ligands switch between various states induced by chemical interactions or conformational changes, while target activation is possible only in a specific state. We find that the activation time is very sensitive to changes of the switching rates, which is a way to modulate cellular signaling. Interestingly, target activation can be fast although the ligand spends most of the time in a non-activating state, which is relevant if activation occurs in a state where the ligand is also prone to degradation. Using a modeling approach and data from FRAP and single particle tracking experiments, we study the switching dynamics of the positive transcription elongation factor b (P-TEFB) inside the nucleus and unravel a novel mechanism of gene regulation. P-TEFB is necessary for the activation of many genes and its motion is controlled by various chemical interactions that alter the state of P-TEFB and its affinity for the DNA.

374-Pos
A Genome-Wide Analysis of Poised Promoters in Bacteria
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As the first and usually rate-limiting step of transcription initiation, bacterial RNA polymerase binds to double stranded DNA (the closed complex formation) and subsequently opens the two strands of DNA (the open complex formation). Poised promoters in bacteria are sequences where RNAP binds with high binding affinity, but which do not have detectable levels of transcription initiation due to too slow transition from closed to open complex. Existence of a considerable number of poised promoters in genome has been often hypothesized, but poised promoters have not been systematically studied, since a large scale analysis of promoter kinetics is not experimentally feasible. To computationally address promoter poising on a genome-wide scale we use a recently developed biophysical model of transcription initiation [1]. We show that promoter poising is significantly reduced by i) Existence of −35 box interactions ii) Binding specificities of (physically independent) RNAP domains that interact with −10 box single-stranded and double-stranded DNA. We show that the later (dominant) effect is not due to generic properties of protein-DNA interactions, and argue that RNAP is designed to reduce promoter poising in genome. However, despite this reduction, we obtain that the number of poised promoters is still significant, and corresponds to ~30% of strongly bound sequences in bacteria [2]. This number roughly matches with lower bound of reported false positives in RNAP ChIP-chip experiments, which suggests that poised promoters are a major contributor to false positives in searches of bacterial promoters.
[1] M Djordjevic and R Bundschuh, *Biophysical Journal* 94: 4233 (2008).[2] M Djordjevic, submitted (2009).

Protein-Nucleic Acid Interactions I

375-Pos
Dynamic Investigation of DNA Bending and Wrapping By Type II Topoisomerases
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Type II topoisomerases catalyze DNA decatenation and unwinding which is crucial for cell division, and therefore type II topoisomerases are some of the main targets of anti-cancer drugs. A recent crystal structure shows that, during the catalytic cycle, a yeast type II topoimerase can bend a 34 base pair DNA segment by up to 150 degrees. Bacterial gyrase, another type II topoisomerase, can wrap an approximately 100 bp DNA segment into a tight 180 degree turn. Bending a stiff polymer like DNA requires considerable energy and could