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.
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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.
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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