the promoter regions control the sensitivity of transcriptional induction as a function of NF-kB concentration.
By using a combination of quantitative biochemical measurements, we show that the NF-kB nuclear concentration is always lower than the dissociation constant for NF-kB binding to specific sites on DNA. We integrate these measurements in a minimal model of a promoter containing multiple sites, based on the thermodynamics of the one-dimensional Ising model. We demonstrate that in the measured NF-kB concentration range the model accounts for the observed analogical transcriptional response under the assumptions that NF-kB binding to adjacent sites is not cooperative, and that bound NF-kB molecules recruit the transcriptional machinery in an additive fashion, each NF-kB interacting weakly but additively with the Pol II complex.

1553-Pos Board B397
Information Processing In Single Yeast Cells: Homogeneous Signal Transduction Result In Heterogeneous Gene Expression
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How cells sense their environment using signal transduction pathways and respond to environmental changes by regulating gene expression is a key problem in systems biology. The mitogen-activated protein kinase (MAPK) pathways, which are evolutionarily conserved from yeast to mammals, provide an excellent model to study how signal transduction is coupled to gene expression. Our research focus on the high-osmolarity glycerol (HOG) MAPK pathway in single, Saccharomyces cerevisiae yeast cells. During the last few decades, the components and regulatory network of this pathway have been elucidated via genetic and biochemical assays performed on large populations of yeast cells. However, surprisingly little is known about the detailed coupling dynamics of signal transduction and gene expression in individual cells. After osmotic shock, homogeneous Hog1 kinase dynamics were measured in all cells. In the subsequent gene expression of STL1, a gene that encodes for a glycerol proton symporter of the plasma membrane, we observed that one subpopulation of cells exhibits no gene expression at all (OFF-population), whereas another subpopulation of cells show gene expression over a wide range of expression levels (ON-population). Further, the ratio of the two subpopulations of cells remained constant despite changes in osmolyte concentration from 0.3 M to 0.6 M NaCl. To identify the origin of the bi-modality in gene expression, we over expressed specific transcription factors that regulate STL1 - gene expression. After over expression of one specific transcription factor, we observed a mono modal gene expression distribution for STL1. Furthermore, single cell time-lapse experiments, indicate that switching between gene expression levels after subsequent osmotic shocks was random and uncorrelated. These results indicate, that at least one transcription factor is responsible for the bi-modality and stochasticity in gene expression.

1554-Pos Board B398
Negative Feedback Speeds Transcriptional Response-Time In Human Cytomegalovirus
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Upon infection of a cell, viruses initiate a complex gene-expression cascade that may result in productive/lytic infection, abortive infection, persistence, or latency depending on the state, cell-type, and environment of that particular cell. However, the regulatory networks underlying these diverse viral lifecycle fates are typically studied using experiments that are averaged over cell populations, potentially masking the dynamic behavior in individual cells. To understand how these diverse viral lifecycle fates are regulated at the single-cell level, we present a framework for quantitatively determining viral expression dynamics and regulatory circuit architectures in individual living cells using a simplified model of a herpesvirus signal transduction module: the human cytomegalovirus (HCMV) Major Immediate Early (MIE) circuit. The system utilizes time-lapse fluorescence video-microscopy of cells infected with recombinant viruses expressing fluorescent gene-products and quantitative modeling to analyze the resulting single-cell data. The combined computational-experimental approach revealed two previously unseen transient signal-processing characteristics of the MIE circuit: (i) negative feedback within the MIE circuit, counter-intuitively, speeds the gene-expression response-time of the essential viral transactivator gene-product IE2-86 (ii) transcriptional activators implicated in reactivation from latency (e.g. trichostatin A) generate a pulse in IE2-86 gene-expression in single-cells. As predicted by the model, mutational deletion of the MIE negative-feedback loop eliminated both the IE2-86 pulse and the accelerated response-time during viral infection. We propose that the negative-feedback architecture of the HCMV MIE circuit may allow the virus to respond quickly to external signals and outpace cellular innate defenses at the single-cell level.

1555-Pos Board B399
Pitchfork And Hopf Bifurcations In Stochastic Regulatory Networks With Delayed Feedback
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The bifurcation diagram of a model nonlinear Langevin equation appropriate for delayed feedback loops in regulatory networks is obtained. We show that under parametric fluctuation the bifurcation remains sharp, both in the ranges of direct and oscillatory bifurcation. At threshold, the stationary distribution function becomes a power law in the dynamical variable. The combined effect of delay and stochasticity leads to a shift in the location of the bifurcation threshold relative to the deterministic limit of no fluctuation. The shift scales linearly with the noise intensity.

1556-Pos Board B400
Power-Law Scaling in Protein Synthesis of a Stochastic Regulon-An Experimental Study
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We investigate the protein expression pattern of the lamB gene in Escherichia coli bacterium. The gene product LamB is an important membrane protein for maltose transport into cells but it is also exploited by bacteriophage lambda for infection. Using a dual-colored phage labeling technique, we find that the LamB receptor distribution p(n) has a majority population with average receptor number n~500 and a minority population at small n. This small-n distribution is scale invariant with p(n)~ n̂α. A power law is also observed when LamB expression is chemically repressed by growing the bacteria in a glucose medium. We propose a heuristic model which can account qualitatively for our observations.

1557-Pos Board B401
Exploiting stochastic gene expression to infer promoter regulatory mechanisms: The Moment Analysis Method
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We introduce the moment analysis method for single cell gene expression data. This method exploits higher order moments (greater than two) of steady state fluorescence distributions from genetically identical cells expressing fluorescent proteins to make inferences about the regulatory mechanism of expression at the promoter level. We show that moment analysis can discriminate between mechanisms with a different number of kinetically relevant promoter states, characterized by the transcriptional rate at each state. For instance, we can distinguish between promoters that transcribe genes at a constant rate and those that switch between active and inactive states of transcription. We can also estimate values for the parameters that define the mechanism, such as the transcription rate, the translation efficiency, and the rates at which the promoter switches between different states. We apply this method to analyze gene expression data from the Ptet07 promoter in yeast. In our experiments this promoter is placed at different genomic locations within the yeast chromosome, some of them in silenced and others in open chromatin regions. Our analysis indicates that when we place the promoter-gene system at an open chromatin location, the promoter is well modeled by a single state mechanism, in which the gene is transcribed at a constant rate. Analysis of silenced regions indicates a more complex regulatory mechanism, involving transitions between active and inactive states of the promoter.

1558-Pos Board B402
Feedback Control Of Fluctuations In Gene Expression And Epigenetic Memory
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Genes and proteins are organized into extensive networks that allow cells to respond and adapt to their environment. These networks are regulated through feedback loops. The dynamic behavior of a genetic network with a given architecture depends on stochastic fluctuations of the agents involved. However, it's still undetermined how the feedback strength and the noise levels couple to determine cellular behavior and its variability. In the present work, we analyze the stochastic dynamic behavior of the galactose uptake network of S. cerevisiae. We focus on how changing the strength of the positive and negative feedback loops influences circuit dynamics and adaptability, and how feedback controls

the noise levels in the circuit. We show that when the strength of the negative feedback is increased, the capacity to display memory of the initial galactose consumption is lost compared to the wild type strain. On the other hand, by varying the strength of the positive feedback we observe the emergence of a region with stable memory. It has been shown that the capacity for cells to display memory depends on the stochastic fluctuations of the circuit, and hence we analyze the effect of feedback strength on transition rates between the alternative states ON/OFF. In this case we demonstrate that the switching rates between the two phenotypic states can be tuned by changing the strength of the feedbacks. These results reveal that the feedback strengths of the network regulate the dynamic behavior through modulation of the stochastic fluctuations of gene expression and the stability of different states of gene expression. This suggests that the strength of feedbacks may be tuned allowing a population to enhance its fitness under a certain frequency of environmental fluctuations, by changing the rate of stochastic transitions between different states.

1559-Pos Board B403

Nature, Nurture Or Just Blind Chance: Stochastic Gene Expression And Its Consequences

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Life itself is a study in the contrast between randomness and determinism: from the chaos of biomolecular interactions to the precise coordination of development, living organisms are able to resolve these two seemingly contradictory aspects of their internal workings. The traditional means by which scientists reconcile the stochastic and the deterministic is by appealing to the statistics of large numbers, thus diminishing the importance of any one molecule in particular. However, cellular function often involves small numbers of molecules, of which perhaps the most important example is DNA. It is this molecule, usually present in just one or few copies per cell that gives organisms their unique genetic identity.

But what about genetically identical organisms grown in homogenous environments? To what degree are they unique? In this talk I will present experiments on bacteria, yeast and nematodes that suggest that even genetically identical individuals exposed to identical environments can be very different. Moreover, some of the most striking sources of this variability are random fluctuations in the expression of individual genes.

In some cases populations might even exploit these fluctuations to improve their chances of survival in variable environments.

1560-Pos Board B404

DNA Architecture and Transcriptional Regulation Exploring DNA's Mechanical Code

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DNA architecture plays a key role in determining spatial and temporal patterns of gene expression. This architecture encompasses both the nucleotide sequence (i.e., the information content) and the physical state of the DNA such as its spatial organization and mechanical properties. We explore transcriptional regulation by DNA looping in the *lac* operon, where transcriptional control is realized by the simultaneous binding of Lac repressor to two binding sites separated by hundreds of base pairs on the DNA. We develop a statistical mechanical model to quantify repression and the *in vivo* energy cost of different DNA conformations in bacteria.

Based on the falsifiable predictions generated by this model we construct a library of promoters in which their DNA architecture is varied systematically. Properties such as the length of the intervening DNA and its sequence-dependent flexibility are controlled and their resulting effect on the gene expression level and its noise are quantified at the single cell level. The goal of this work is to make a systematic and thorough comparison of theory and experiment in a parameter-free setting which strictly tests our understanding of the relation between DNA architecture and the level of gene expression.

1561-Pos Board B405

A Study of Cro's Role in the Induction of Phage Lambda Switch by Stochastic Probability Landscape Model

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The genetic switch of phage lambda is controlled by the double negative feedback loop of CI and Cro. Although, Cro as a repressor of the PRM promoter for CI has been studied for several decades, the role of Cro in phage lambda lytic development has not been fully understood. Evidence indicates that Cro help the induction of phage lambda by turning down lytic transcription via the binding of operator OR1 and OR2 at PR promoter and repressing the PRM promoter for CI via the strong binding at OR3. To investigate which binding of Cro is critical in the induction of phage lambda, we compute the exact steady state probability landscape of the genetic circuit of the switch network. We demonstrate that the reduction of binding affinity of Cro on OR3 has elongated the lysogenic state and strongly inhibited the transition from lysogeny to the lytic pathway which is in good agreement with the mutations studied by Schubert et al. in 2007. The stability and sensitivity of phage lambda switch and its robustness are also analyzed in our study.

1562-Pos Board B406

Cellular Particle Dynamics Simulation Of Bioprinted 3d Tissue Constructs

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Previous studies have shown that under certain conditions living tissues and multicellular aggregates behave as highly visco-elastic liquids. Tissue liquidity, brought about by cellular adhesion and motility, forms the basis of the newly developed bioprinting technology, which is used to design and build 3D tissue constructs by employing computer-controlled layer-by-layer deposition of bio-ink (submillimeter size cell aggregate) droplets onto biopaper (biocompatible gel). In order to describe and predict the self-assembly process of bioprinted multicellular constructs we have developed a computer simulation method referred to as cellular particle dynamics (CPD). In CPD cells are modeled as an ensemble of cellular particles (CPs) that interact via short range contact interactions, characterized by an attractive (adhesive interaction) and a repulsive (excluded volume interaction) component. The time evolution of the spatial conformation of the multicellular system is determined directly by recording the trajectories of all CPs through integration of their equations of motion. The cellular level CP model parameters are related to the experimentally measurable tissue level biophysical quantities (e.g., surface tension, viscosity and shear modulus) by comparing the results from selected benchmark experiments (e.g., compression and fusion of spherical cell aggregates) with those from the corresponding CPD simulations. Here we apply the CPD method to describe and predict in silico the post-bioprinting time evolution of the formation of tubular multicellular structures (which resemble primitive blood vessels). Our CPD simulations take substantially less time and effort than the corresponding experiments and, most importantly, provide results in good agreement with the experimental ones.

Work supported by the National Science Foundation [FIBR-0526854]. Computer time provided by the University of Missouri Bioinformatics Consortium.

1563-Pos Board B407

Inversion of Membrane Protein Gating Models in Bioelectricity

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In recent years we have seen a dramatic increase in the complexity of Markov model configurations. While single protein data allow to directly estimate the Markov transition rates from the data it is not possible to do so with macroscopic data. In several instances experimental constraints does not permit single protein measurements. This limitation combined with the complexity of Markov model configurations makes the estimation problem a non-trivial one.

Here we address the task of finding the Markov rates from macroscopic data. We assume the transition rates functions of one independent variable (e.g. the membrane voltage). We do not constrain the dependence to any particular form. Indeed the dependence of the transition rates with respect to the independent variable is represented with Bsplines. The method we introduce is truly non-linear. The Bspline coefficients are obtained applying a sequence of non-linear transformations to the data.

Set of currents obtained in voltage clamp stimulation protocols (or clamp of the independent variable) are represented by exponential time series. We first introduce a generalization of Prony's method that allow to obtain unambiguously the coefficients and arguments of the exponential time series associated to each current. We show that the estimation of the Markov rates from the coefficients and arguments of the exponential time series constitutes an inverse eigenvalue problem. We introduce a procedure that allows to solve this eigenvalue problem with a sequence of nonlinear transformations. We apply the method to currents produced by a given Markov model which allows us to judge the accuracy of the procedure. An interesting outcome of our analysis is that the Markov rates are not unique if a minimal and complementary data set is not produced.

1564-Pos Board B408

Gap Junction Adaptation as a basis of cardiac memory - A computational Study

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