potentials. Disulfide redox potentials measured in thiol-disulfide oxidoreductases range from -120mV to -270mV. For disulfides serving structural purposes, the redox potential can be as low as -470mV. However individual measurements of this kind are difficult and time consuming. Computational approaches that can identify and characterize redox active disulfides will contribute significantly to our understanding of disulfide redox-activity.

Seminal studies by Richardson and Thornton defined the constraints imposed by protein structure on disulfide formation and flagged forbidden regions of primary or secondary structure seemingly incapable of forming disulfide bonds between resident cysteine pairs. With respect to secondary structure, disulfide bonds were not found between cysteine pairs:

– A. on adjacent β-stands;
– B. in a single helix or strand;
– C. on non-adjacent strands of the same β-sheet.

In primary structure, disulfide bonds were not found between cysteine pairs:

– D. adjacent in the sequence.

Here we identify nine different types of disulfides that occupy these forbidden regions. Most have high torsional energies, a quantity that has been related to the ease with which a disulfide can be reduced. It has been observed that sources of strain in a protein structure, such as residues in forbidden regions of the Ramachandran plot and *cis*-peptide bonds, are found in functionally important regions of the protein and warrant further investigation. Here we show that many of these "forbidden" disulfides act as redox-regulated switches of protein function.

Regulatory Networks & Systems Biology - II

3289-Pos Smooth turning mechanism of crawling *C. elegans*

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Board B592

Caenorhabditis elegans (*C. elegans*) is the model organism with relatively simple anatomy and well characterized genetic information. There has been a great deal of efforts to understand the mechanism of *C. elegans* locomotion. When crawling on a solid surface, *C. elegans* moves forward and backward by propagating dorso-ventral contraction waves toward the opposite direction of its movement, the mechanisms of which have been extensively analyzed through mechanical and neural modeling. In these studies their simple straight motions are mainly considered while the turning mechanism in crawling is mostly neglected.

In this research, we propose a simple mathematical model for the turning of crawling *C. elegans*. It reveals that the worm regulates the bending curvature and interval between ridges of its muscular waves during the turns. These regulations lead to the changes of two major motion parameters, namely the ratio of amplitude to wavelength and body length normalized wavelength. The proposed model indicates that the worm is able to turn by causing the changes in these two parameters, which is consistent with what we observe in experiments.

3290-Pos Nf-kappaB Subunit P65 Antagonizes The Nrf2-are Pathway By Depriving Cbp From Nrf2 And Facilitating Recruitment Of Hdac3 To Mafk

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Board B593

Constitutively activated NF-κB occurs in many inflammatory and tumor tissues. Does it interfere with anti-inflammatory or anti-tumor signaling pathway? Here, we report that NF-κB p65 subunit repressed the Nrf2-antioxidant response element (ARE) pathway at transcriptional level. In the cells where NF-κB and Nrf2 were simultaneously activated, p65 unidirectionally antagonized the transcriptional activity of Nrf2. In p65-overexpressed cells, the ARE-dependent expression of heme oxygenase-1 was strongly suppressed. Experiments with luciferase reporters containing AREs of various anti-inflammatory genes showed that overexpression of p65 significantly inhibited the Nrf2-mediated ARE-driven gene expression, while knockdown of the endogenous NF-κB obviously enhanced the Nrf2-mediated gene expression. However, it was found that p65 inhibited the ARE-driven gene transcription in a way that was independent on its own transcriptional activity. Immunoprecipitation analysis showed that overexpression of p65 inhibited, but knockdown of p65 enhanced the association of CREB binding protein (CBP) to Nrf2. Chromatin immunoprecipitation study showed that overexpression of p65 also increased the MafK-associated histone deacetylase 3 (HDAC3) and reduced acytilation of histone H4 in chromatin level.

In summary, two mechanisms were found to coordinate the p65-mediated repression of ARE:

1. p65 selectively deprives CBP, the co-activator of Nrf2, from Nrf2 by competitive interaction with the CH1-KIX domain of CBP, which results in inactivation of Nrf2. The inactivation depends on PKA catalytic subunit-mediated phosphorylation of p65 at S276.
2. p65 promotes recruitment HDAC3, the co-repressor, to ARE by facilitating the interaction of HDAC3 with either CBP or MafK, leading to local histone hypoacetylation.

This investigation revealed the participation of NF-κB p65 in the negative regulation of Nrf2-ARE signaling, and may provide a new insight into a possible role of NF-κB in suppressing the expression of anti-inflammatory or anti-tumor genes.

3291-Pos Bioavailability of VEGF in the extracellular matrix: Mathematical models

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Board B594

Vascular Endothelial Growth Factor (VEGF) is a critical molecule in the process of tumor-induced angiogenesis, being a promoter of endothelial cell proliferation and migration. The extracellular matrix (ECM) is known to be a reservoir of VEGF, as VEGF can bind to heparin sulfate proteoglycans (HSPGs) in the ECM. We use mathematical and computational techniques to study the regulation of VEGF bioavailability by ECM remodeling and proteolytic cleavage. We show that ECM-binding VEGF isoforms (e.g. VEGF165 and VEGF189) can be available to endothelial cells before the "angiogenic switch" if there is either a low density of HSPG binding sites for VEGF or a high level of endogenous VEGF in the ECM. The release of MMPs during the angiogenic switch, and subsequent proteolytic cleavage, serves to regulate the spatial distribution of ECM-bound VEGF, and is important in giving rise to the different vascular phenotypes associated with the various VEGF isoforms.

However, whether the resulting concentration profile (of ECM-bound VEGF) depends on the details of the VEGF-HSPG binding sites, as well as the extent of matrix-remodeling during the angiogenic switch. If the VEGF fragments left behind by proteolytic cleavage continue to occupy binding sites without being cleared from the ECM, the concentration of ECM-bound VEGF will decline over time. We identify the crucial rates and time scales necessary to give rise to different qualitative effects. Finally, we identify open questions regarding how the availability of VEGF is regulated by interactions with the ECM, and implications for anti-angiogenic therapies.

3292-Pos Dynamic Patterns of NF-λB Translocation due to Competitive Interplay Between Transcriptional Delay and Noise in NF-λB Signaling Network

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Board B595

NF-κB is a stimulus-responsive pleiotropic regulator of gene control. A single NF-κB signaling network is able to regulate multiple cellular functions such as differentiation of immune cells, cell apoptosis, and immune activation. Understanding how cells achieve cell type-specific, context-specific, and stimulus-specific responses by employing a single NF-κB signaling pathway in so many functions is a great challenge. One of running hypotheses is that the dynamic patterns of NF-κB shuttling between cytoplasm and nucleus must be responsible for the pleiotropism of NF-κB. Our work was motivated by David Nelson *et al.* [Science *306*:704 (2004)], which showed noisy periodic oscillations of NF-κB translocation between cytoplasm and nucleus in a single cell. We investigated the oscillatory dynamics of NF-κB translocation with a stochastic two-compartmental (cytoplasm and nucleus) model, especially taking into account the competitive interplay between noisy transcription and transcriptional delay. Unlike stochastic systems with a single delay where delay and noise synergistically enhance the oscillatory behavior, the NF-κB signaling network under our consideration involves multiple negative feedback loops with different transcriptional delays suppressing the propensity of oscillation. Using both stochastic simulations and analytical approaches, we will provide the resulting dynamic patterns of the NF-κB translocation due to competitive interplay between stochastic transcription and multiple negative feedback loops with different time-delays.

3293-Pos Exact Stochastic Probablistic Landscape of Molecular Networks in Systems Biology without Gillespie or Fokker-Planck: Critical Molecular Events in Genetic Switch of Prophage Induction of Phage Lambda

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Board B596

Many biochemical networks in a cell involve molecular species of small copy numbers. A problem of central importance in systems biology is to understand the effects of stochasticity intrinsic in these networks.

The chemical master equation (CME) provides the fundamental framework for this problem. Although Fokker-Planck/Langevin methods provide useful approximations, the Gillespie algorithm is currently the only available method to study the full stochastic nature of such networks via simulation. However, it cannot sample rare but critical events effectively. In this work, we describe a new method to directly solve the CME to account for full stochasticity without either approximations nor simulation for nontrivial systems. We first develop a method to characterize the state space of a molecular network at a given initial concentration condition. In contrast to the defeatist view that this space is astronomically large, we show our method can characterize the state space exactly for many realistic networks, including a model of the MAPK network involving 13 species. We then show how to compute the exact steady state and time-varying dynamic probablistic landscape of all species in the nework. The lysis-lysogeny decision of bacteriophage lambda has been a paradigm for understanding developmental genetic network. We apply our method to study the critical molecular events in the genetic switch of phage lambda regulatory network, and establish detailed stochastic mechanisms of lysogenic induction.

3294-Pos Cooperation in yeast sucrose metabolism

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Board B597

The conditions required for the initiation and maintenance of cooperation is a classic problem in evolutionary biology. As a model system to quantitatively test models in evolutionary game theory we are studying the metabolism of sucrose in the yeast *S. cerevisiae*. Digesting sucrose requires that the disaccharide be hydrolyzed into glucose and fructose, a reaction which is catalyzed by the enzyme invertase in the periplasmic space between the plasma membrane and the cell wall. We have shown both theoretically and experimentally that a majority of the resulting monosaccharides diffuse away before they can be imported into the cell. The hydrolysis of sucrose is therefore a cooperative behavior.

We have characterized the wild-type invertase production strategy by inserting YFP under the control of the invertase promoter. A low basal level of invertase expression coupled with an increase in expression at low glucose concentrations acts as a "sensor" of sucrose. In sucrose media wild-type cells initially turn ON expression of invertase, but as the culture grows the concentration of glucose in the media increases to the point where invertase expression is repressed. The wild-type invertase production strategy is therefore a dynamic function of the environment.

We have competed the wild-type cooperator against a mutant "cheater" strain that is unable to produce invertase. We use a histidine auxotroph cooperator, thus allowing us to control the "cost of cooperation" by varying the concentration of histidine in the growth medium. In a well-mixed environment we find that cooperators and defectors coexist, consistent with the interaction being a snowdrift game, in which the optimal strategy is the opposite of what one's opponent is doing. Finally, we probe the success of the cooperator in spatially-structured competition and find that the effect depends upon the precise nature of the spatial structure.

3295-Pos Mathematical Modeling Of Cellular Signaling - Master Equation And Generalized Stochastic Simulation Algorithm (ssa) Based Approach

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Board B598

We have developed a master equation and stochastic differential equation based approach to study cellular signaling processes. Master equation formalism has been used heavily to study random walk processes. We discuss how such master equation formalism can be used to model biochemical reactions in cellular signaling networks. We can readily derive the probability distribution of different signaling species directly from the solution of the master equation. We also discuss the stochastic differential equations associated with master equations and a generalized scheme of solving Gillespie stochastic simulation algorithm that includes spatial diffusion.

3296-Pos Oscillation-based Apoptosis: Why A Biologist Can Fix A Radio

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Board B599

The tumor suppressor protein p53 is activated in response to cellular stress, and coordinates the actions of several downstream processes, including DNA repair, cell cycle arrest and apoptosis. At the single cell level, the p53 response to ionizing radiation was originally characterized as "digital pulses" of relatively fixed amplitude and period lasting up to several days, with the level of DNA damage encoded by the number of pulses that could be counted by downstream processes. More recent analysis of the stress induced p53 signal, however, has revealed significant cell-to-cell variability, but with clear spectral content at ~6 hr. period. To use this signal, the cellular machinery must detect the amplitude in this narrow frequency band while ignoring higher frequency noise. This is especially important in apoptosis, a tightly regulated, genetically controlled process of cell death activated in response to developmental cues, cellular stress or infection, and known to play a key role in development, homeostatis and disease. In the spirit of Lazebnik's "Can a biologist fix a radio?–Or, what I learned while studying apoptosis" here we present and analyze a mathematical model of apoptosis signaling based on the concepts of heterodynes and lock-in amplifiers, and demonstrate how a process downstream of p53 signaling might detect and count digital p53 pulses in the presence of significant variability.

3297-Pos The Brownian flip flop, from deterministic dynamics to deterministic logic

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Board B600

Allosteric signaling networks (ASN)—semi-stable assemblies of proteins that communicate using localized information in the form of coupled domain movements—are the principal devices used by the cell to processes intracellular and extracellular chemical information. The regulatory apparatus of cardiac muscle is a prototype ASN. It consists of the Ca-sensitive troponin C (TnC), troponin I (TnI), troponin T, tropomyosin (Tm), seven polymerized actin and myosin (collectively called acto-myosn, AM). The molecular components can be parsed into discrete modules, or quaternary-level protein complexes—the Calcium regulatory switch (CRS), consisting of TnC, TnI, TnT and Tm; and the AM switch (AMS), consisting of Tm and AM. The regulatory apparatus is viewed as a penenary-level

structure consisting of interacting the quaternary-level protein modules: CRS and AMS. The free energies in of the lowest energy system-states form the "system phase free-energy landscape" (SPEL) of the network. The SPEL offers critical insight into the function of an ASN because using the Boltzmann equation, the SPEL determines the equilibrium distribution of system-states at any [Ca].

From time-resolved FRET, stopped flow FRET, and FRET-Ca-titration experiments we have determined the SPEL of the CRS. The SPEL of the AMS module is constructed from previously reported measurements. The regulatory apparatus is a back-to-back construct of the CRS with the AMS that is of similar design but inverted shape. The design, called the Brownian flip flop (BFF), achieves high sensitivity, high specificity, and high switching speed using modules, which as isolated units, function poorly. The BFF architecture is recognized by features that enable its function—a plateau flanked by receptors that alternate between network attractors and network repellers according to their liganded state. The assembly functions as a Browniain computer that performs an "equivalence" logic operation. Its function is isomorphic with its structure.

3298-Pos 2D Computational Model Simulation of ROS-induced Mitochondrial Network Oscillation

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Board B601

Previous studies have revealed that reactive oxygen species (ROS) release and mitochondrial membrane potential ($\Delta\Theta_m$) depolarization in less than 1% of the cellular volume can trigger spatiotemporally synchronized cell-wide oscillations (Aon et al., 2003). How the highly localized perturbation of a few mitochondria triggers the global self-organized spatiotemporal behavior is incompletely understood. We hypothesized that synchronization occurs cooperatively from initially depolarized mitochondria which then recruit their neighbors through ROS diffusion and ROS-induced ROS release, amplifying the initial mitochondrial event and eventually causing synchronized cell-wide oscillation. To test the hypothesis, a 2D computational model of the network was developed which is comprised of a Mitochondria Energetics - ROS- induced ROS release (ME-RIRR) model (Cortassa et al., 2004) arranged in a lattice. The ME-RIRR model incorporates major components of mitochondrial energy metabolism, ROS dynamics and a ROS-activated inner membrane anion channel. Oxidative stress was induced in the model by increasing mitochondrial ROS production to trigger $\Delta\Theta_m$ depolarization in a fraction of the network, and the influence of ROS diffusion on $\Delta\Theta_m$ wave propagation was studied by changing the diffusion coefficient. The model successfully reproduced the $\Delta\Theta_m$ depolarization wave observed in experiments in response to oxidative stress. Increasing the ROS diffusion coefficient significantly increased wave velocity. Remarkably, a few oscillating mitochondria could entrain the whole network into an oscillatory mode even though the parametric space of the majority of the mitochondrial lattice was not in the oscillatory domain. In accordance with critical phenomena, model simulations demonstrate that due to interdependent network behavior, perturbing a minimal number of mitochondria is sufficient for ROS to propagate and trigger the cell-wide synchronized oscillation.

3299-Pos Results from a Novel Cellular Dynamics Simulator Reveal a Quantitative Mechanism for NMDA Receptor-Dependent CaMKII Translocation in Dendritic Spines

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Board B602

Particle-based Monte-Carlo simulations are an important tool for the analysis of microscopic molecular physiology. One of the major challenges in the field is how to accurately simulate molecular diffusion, interaction, and multi-protein complex assembly in the cellular environment. Here we present a novel event-driven simulation scheme (Cellular Dynamics Simulator, CDS) that provides maximum flexibility of the algorithmic scheme to simulate molecular interactions from the single molecular level to the cellular level.

Using this simulator, we investigate intracellular translocation of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) in dendritic spines of hippocampal CA1 pyramidal neurons. The translocation of CaMKII from cytoplasm to the post-synaptic density (PSD) requires Ca^{2+} entry through NMDA receptors but not from other sources of Ca^{2+}. Subsequent activation of CaMKII by Ca^{2+}-calmodulin (CaM) and/or autophosphorylation promotes and stabilizes the interaction of CaMKII with NR1, NR2A, and NR2B subunits of the NMDA receptor.

Quantitative understanding of such a process requires explicit modeling of molecular diffusion in three-dimensional space, chemical transitions between a large number of activation states, and assembly of multi-protein complexes. We investigate the impact of CaM concentration, the spatial distribution of NMDA receptors, and the duration/amplitude of Ca^{2+} transients on the kinetics of CaMKII translocation to the NMDA receptor. Specifically, we hypothesize the limited amount of CaM in dendritic spines results in a time delay in Ca^{2+}/CaM activation and in autophosphorylation of CaMKII. This may explain why spatially restricted and prolonged high-Ca^{2+} transients mediated by NMDA receptors are necessary for efficient CaMKII translocation. The simulation results are also compared to recent experimental data and we confirm the role of CaMKII autophosphorylation in the translocation.

3300-Pos Waiting Time Distributions For Clusters Of Complex Molecules

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Board B603

Waiting time distributions are in the core of theories for a large variety of subjects from the analysis of patch clamp records to

stochastic excitable systems. Here, we present a novel exact method for the calculation of waiting time distributions for state transitions of complex molecules with independent subunit dynamics. The absorbing molecule state couples the subunit dynamics in waiting time calculations. The subunits can posses an arbitrary number of states and any topology of transitions between them. The method circumvents problems arising from combinatorial explosion due to subunit coupling and requires solutions of the subunit master equation only.

3301-Pos Tumor Necrosis Factor Receptor-2-Mediated Tumor Suppression is Nitric Oxide Dependent and Involves Angiostasis

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Board B604

Tumor necrosis factor (TNF) binds to two different receptors. While most of its functions are contributed to TNF receptor (TNFR) 1, the independent role of TNFR2 is still largely unknown. Using TNFR single or double knockout mice, we show here that the expression of TNFR2 alone on host cells was sufficient to suppress the growth of TNF secreting tumors in both immune competent and T/B lymphocyte-deficient SCID mice. Histological studies showed that TNF recruited via TNFR2 large numbers of macrophages and efficiently inhibited angiogenesis in the tumor. In vitro, TNF activated TNFR1-deficient macrophages to produce nitric oxide (NO). Treatment of TNFR1 knockout mice with L-NAME, a specific NO synthase inhibitor, almost completely eliminated TNF-induced angiostasis and tumor suppression. Moreover, L-NAME acted only during the first few days of tumor growth. Our results demonstrate for the first time that TNFR2 expressed on host innate immune cells is sufficient to mediate the anti-tumor effect of TNF and NO is necessary for this process, possibly by inhibition of angiogenesis in the tumor.