networks through a set of biochemical reactions is generally thought to predict an average kinetic behavior. Stochastic kinetic methods, accounting for random fluctuations, become essential to characterize intrinsic heterogeneity due to low copy numbers and noisy environments present in these systems. While in certain regulatory constructs, it has been shown that a fully discrete stochastic treatment of fluctuations through the Chemical Master Equation yields multi-stability when deterministic treatment predicts monostability; deterministic or continuous approaches continues to be the most widespread method for modeling the behavior of cell populations. We explore stochastic variability in cellular dynamics through a varied treatment of intrinsic noise, the randomness arising either at the level of a single reaction, single molecule or as a drift in concentration of the involved species. Our study involves modeling of a genetic toggle switch which is a set of two mutually repressing genes, acting as a cellular ''memory device'' during cell differentiation process, deciding the final cell fate. We simulate the complex switching dynamics between the multi-attractor states in these systems through direct sampling using continuous stochastic approaches Langevin dynamics and Fokker Plank equation as well as discrete stochastic methods Gillespie's Stochastic Simulation Algorithm and numerical solution of the Chemical Master Equation. The stochastic trajectories obtained through numerical simulations not only unveil the complex dynamics leading to multi-stability, they also help explore rigorously the variations, if any, in the dynamics of transitions. Our results can further be used to compare the statistics of switching events and hence to benchmark the various computational methods available to model stochasticity in such systems.

1572-Pos Board B523

Optimizing Protein Expression Levels as a Function of Network Topology Minimizes Nonfunctional Complex Formation

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Human cells contain ~20,000 genes encoding upwards to 100,000 protein types. 5-40% of cell volume is occupied by macromolecules, posing challenges for cell proteins to locate functional partners and avoid nonspecific interactions. Overexpressed proteins may saturate functional partners, leaving leftovers for nonspecific binding instead. Eukaryotic cells have evolved various methods to help proteins function reliably, including compartmentalization, allostery, and structural properties of binding sites. In addition to these, cells may optimize protein expression levels as a function of network topology to avoid nonspecific binding.

To study the effects of relative protein abundance on nonspecific complex formation, we first simulated five simple network motifs under varying protein concentrations using the Gillespie algorithm. While the motifs formed roughly the same proportion of nonspecific interactions under optimal conditions, they varied in sensitivity to initial concentrations (ICs), with the hub being the most sensitive and triangle being the least. We then simulated 500 large networks of 90-200 nodes with varying topological properties under equal, random, and optimized ICs. Binding affinities for all specific and nonspecific interactions were determined using a coarse-grained protein sequence model. The proportion of protein in nonspecific complexes was recorded as a function of degree distribution, network density, average binding strength, local topology, and ICs. It was found that optimizing the local topology via introducing more hubs and less chains and flags; similar to real networks; decreased the number of nonspecific complexes under optimal ICs, but also increased sensitivity to ICs. Degree distribution, surprisingly, had little influence once local topology was optimized. We conclude that there is evolutionary pressure to both favor certain network motifs and to balance protein abundance to avoid misinteractions.

1573-Pos Board B524

Designing Stem-Cell Based Anti-HIV Therapies using Molecular-Detailed Multiscale Models

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Although HAART ensures normal lives for HIV-infected individuals, it does not lead to a functional cure. In 2008, the ''Berlin patient'', a HIV-infected cancer patient, underwent a bone marrow transplant from a CCR5-/- donor. Since then, he has shown no signs of active HIV-1 replication in the absence of HAART. However, finding such a matched donor for each HIV patient is challenging; instead, inserting anti-HIV genes into patients' or matched donors' stem cells before transplantation could provide HIV-resistance to the progeny target cells and lead to a functional cure.

We developed a molecular-detailed, mechanistic model of HIV infection to design stem-cell therapies using endogenous anti-HIV genes and to identify logistics of successful treatment. The model simulates the dynamics of HIV infection to track key restriction factors and their interactions with HIV-encoded proteins, the virus, and multiple immune cell types: CD4+ T-cells, macrophages, latently-infected T-cells, and CTLs. Using viral load and T-cell count data from HIV-infected individuals, we explored interpatient variability of stem-cell therapies by creating a virtual population of HIV patients. Our model predicted that APOBEC3G overexpression at medium or high transduction efficiency could effectively stop HIV replication and increase the T-cell count to normal levels. SAMHD1 overexpression in macrophages even at high transduction efficiency is not by itself a potent inhibitor of HIV replication; it stabilizes the viral load and slightly improves the T-cell count. However, the model predicted APOBEC3G-SAMHD1 synergy: faster viral decay and return to normal T-cell counts at lower overexpression levels. Surprisingly, HIV-triggered pro-apoptosis gene circuits combined with APOBEC3G overexpression were predicted to negatively impact HIV replication blockade if expressed at low transduction efficiency. This is because some infected T-cells die at a faster rate, weakening the effect of immune response in killing the remaining infected cells.

1574-Pos Board B525

DNA Fluorescence Parameters and Methylation Levels of Gut Commensal Escherichia coli from Crohn's Disease Patients

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Previously it was shown by us the high concentrations of E. coli in feces of Crohn's disease (CD) patients. The differences in growth of gut commensal E. coli both in aerobic and anaerobic conditions, was also demonstrated [1, 2]. Taking into account the preliminary results on DNA methylation levels and the possible impact of DNA structure on DNA fluorescence parameters, the goal of current investigations was to study the DNA fluorescence parameters of commensal E. coli isolates from the gut microbiota of CD patients in association with the DNA methylation levels.

10 CD patients and 10 healthy persons were involved in this study, and at least, 5 randomly selected gut E. coli isolates from each healthy and diseased person were investigated.

Comparative analysis has shown that DNA methylation levels in patients' E. coli were significantly higher than in E. coli of healthy individuals, and the levels correlated with the duration and stage of the disease. The differences in fluorescence parameters of DNA from CD patients' gut E. coli isolates were also revealed by us compared with the control samples of healthy people. Chronic inflammation induces a cascade of pro-inflammatory and anti-inflammatory molecules. The balance between these two groups of regulators controls cell death and repair of tissue damage. In recent years it has become apparent that gut commensals produce molecules which can counteract pro-inflammatory and anti-inflammatory pathways leading to activation or repression of immunity genes.

The above mentioned data suggests that epigenetic gene control mechanisms can be involved in bacterial induced or supported inflammation during CD.

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1575-Pos Board B526

Isolation, Fragmentation and the Detection of Listeria DNA from Ground Beef

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Listeria is a gram-positive, rod shaped food borne bacterial pathogen with a mortality of 20-30% of those who get infected. People with a compromised immune system and pregnant women are more likely to suffer effects of Listeriosis.[1] Listeria bacterium can grow under extreme conditions such as low pH and high temperatures. Places that are most likely to transmit the bacterium are food processing plants during any food processing step. Once Listeria is contracted in the human body, it can cross in to the blood, through the blood brain barrier, and into the cerebral spinal fluid. Current detection such as PCR methods are slow and costly and the extraction methods to see if Listeria is present is blood work, spinal tap, or a biopsy of the placenta.

In this paper we show that Listeria DNA can be efficiently and rapidly extracted from ground bovine meat and lysed. Our microwave based lysing approach[2] has particular advantages in that it can fragment the Listeria genome to smaller

DNA fragments, which is ideal for rapid detection using the MAMEF (Microwave-Accelerated Metal-Enhanced Fluorescence) platform[3], as well as diminishing the effects of DNases due to the elevated lysing temperatures.

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1576-Pos Board B527

Rapid Microbial Lysing and DNA Fragmentation by Microwave Focusing

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Bacterial infections are a major health problem worldwide. Identification of disease-causing organisms by culture-based approaches is time-consuming and often lacks sensitivity. Molecular approaches such as PCR and microwave-accelerated metal-enhanced fluorescence (MAMEF) assays1, are more sensitive and faster than traditional culture-based approaches, but require isolation of the target DNA. In order to determine the effect of both boiling and microwave irradiation on microbial lysing and DNA fragmentation, cultures of Neisseria gonorrhoeae and Listeria monocytogenes (108 CFU /mL) were either boiled (range 40° - 70°C) or lysed in a 900-watt microwave on isolator-mounted microscope slides, both with and without the assistance of disjointed antenna gold bow-tie structures. The temperatures of cultures were obtained prior to and after lysing and the resulting lysate cultured on selective agar plates. DNA isolation and fragmentation efficiency were determined by gel electrophoresis and PCR. N. gonorrhoeae lysed at a lower temperature (°C) than L. monocytogenes. Microbial lysing and DNA fragmentation was more effectively carried out in the presence disjointed gold triangle structures, but only when small sample volume were used. Standard boiling was successful for bacterial lysing and DNA fragmentation, but required higher temperatures and longer times than microwave focusing. PCR results suggest that low power microwave irradiation is ideal for PCR methods while higher microwave powers are required to generate DNA fragments ideal for MAMEF analysis. Microbial lysing and DNA fragmentation can be achieved by either boiling or microwave, but microwave lysing is more efficient for DNA fragmentation and is significantly faster. Microwave lysing is the recommended method when rapid isolation and DNA fragmentation is required.

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1577-Pos Board B528

Palmitate Re-Directs Glucose Utilization in Type 2 Diabetic Hearts, Improving Function: A Metabolomic-Fluxomic Study

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Hyperglycemia and hyperlipidemia are two main traits of type-2 diabetes (T2DM). T2DM patients may develop a cardiomyopathy, and the excess in nutrients greatly contributes to systolic and diastolic dysfunction. The Randle cycle postulates that fatty acid (FA) utilization further impairs glucose utilization, impeding its oxidation. Yet recent evidence suggests that, when acutely infused, FAs such as palmitate (Palm) actually help in maintaining function in T2DM hearts stressed with high glucose and catecholamines. Thus, under conditions of sustained stress, lipids may be necessary to maintain function in stressed T2DM hearts. Using a novel procedure for translating metabolomics into metabolic fluxes, here we tested whether Palm is able to redirect the glucose fluxome in T2DM hearts, contributing to a better utilization/oxidation of glucose. We found that Palm, without inhibiting glycolysis, led to a 50% increase in glucose oxidation via the pentose phosphate [PP] pathway. Palm presence shifted the control of the glycolytic flux from phosphofructokinase to glucose uptake, glucose 6-phosphate dehydrogenase and glycogenolysis. Palm-induced remodeling of the glucose fluxome decreased the intracellular levels of glucose by 17-fold, owing to reduced uptake at maintained utilization. Moreover, it augmented the content of reduced GSH, via higher NADPH generation through the PP pathway. Our study provides a mechanistic explanation to the in vitro observation that FAs such as Palm are necessary for the T2DM hearts to maintain function when in presence of hyperglycemia and/or increased workload, by remodeling glucose utilization leading to a higher supply of reducing equivalents to the heart. Present findings suggest that in T2DM subjects the Randle cycle may apply to some but not all pathophysiological contexts.

1578-Pos Board B529

Modeling Host - Bacterial Biofilm Interactions in Lower Leg Chronic Wounds

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Chronic wounds are caused by a healing process often stalled at the inflammation, proliferation stages of repair. Each chronic wound displays its own dynamics in a complex 3-dimensional interplay of repair against a range of obstructing factors. These may include host and bacterial genetic makeup, medical and environmental conditions, counter-productive habits and the presence of an opportunistic poly-bacterial biofilm. Currently there is a rapidly growing need to create a treatment plan upon assessment of a wound followed by regular quantitative monitoring. Understanding the spatial and temporal variations in biochemical and biophysical wound parameters will aid a timely healing process. In-silico simulations can support these efforts by modeling the influence of the various factors. Examples are: proper use of smart wound dressings and prevention of the build-up of antibiotic tolerance and resistance.
We present results based on expansions of published models to simulate the destruction of wound bed collagen, the dynamic interplay of host neutrophils, macrophages, fibroblasts and keratinocytes against the detrimental effects of bacterial metalloproteinases (MMPs) under normal, low-oxygen or anaerobic conditions. These simulations also allow to assess the influence of a biofilm even though most of its interactions with host chronic wound-tissue are barely known or understood With growing knowledge computer modeling will aid in management of individual chronic wounds.

1579-Pos Board B530

Model for Aging and Cognitive Decline

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A population of neurons in the cerebral cortex of humans and other mammals organize themselves into vertical microcolumns perpendicular to the pial surface. Anatomical changes to these microcolumns have been correlated with neurological diseases and normal aging, and in particular in area 46 of the rhesus monkey brain the strength of microcolumns was shown to decrease with age. We have previously developed a model to simulate aging brains by constructing a microcolumnar network of neurons and allowing the neurons to undergo Brownian motion while being constrained by a harmonic force that weakens as a function of age. Now, we expand on this model by constructing and simulating the generated neural networks. By generating a young neural network from strong restorative forces, one can create an initial distant dependent connectivity. Then, we age these networks and presume that connectivity between neurons either weakens or severs as a function of neural displacement from initial neuronal positions. We aim to show that older networks are unable to efficiently shift between different firing regimes, providing a potential mechanism for loss of information processing in relation to microcolumnar structure.

Molecular Dynamics II

1580-Pos Board B531

Comparison of Activation Energy and Pore Dynamics in Liquid and Gel Phases of Electroporated Lipid Bilayers using Temperature Dependent MD Simulations

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The molecular level understanding of electroporation has been studied by few research groups [1, 2, 3] over the last decade. We have performed molecular dynamics simulation(MDS) of electroporation at different temperatures to find activation energy as well as pore dynamics in the gel and liquid phases of POPC and DPPC lipid bilayers.
The MDS of bilayers were performed using NAMD, the Particle mesh Ewald(PME) method, the all-atom CHARMM force field and an integrated time-step of 2 fs.
The bilayers were composed of 256 lipids which were solvated with TIP3 water molecules with a low KCl concentration. The MD simulations were performed in temperature range from 250 K to 350 K with varying electric fields (0.02 to 1 V/nm).
A plot of pore initiation rate as a function of inverse temperature showed Arrhenius type behaviour. The activation energy was determined to be 25.5 and