the enzyme activity (i.e., positive feedback). If fluctuations are absent, the system can be described by PDEs with delay. Spatiotemporal patterns such as traveling waves or spirals as well as uniform oscillations are observed.
In this work, we investigate effects of fluctuations in such systems. There are two major sources of fluctuations: conformation of each enzyme fluctuates individually and thus disperses time to finish the cycle (intramolecular fluctuations); stochastic interactions between molecules vary waiting time to start the next cycle (intermolecular fluctuations). Intermolecular fluctuations may in some cases enhance synchronization of the enzymes, while intramolecular fluctuations merely disturb it. We show that the combination of these two types of fluctuations may determine the dominant spatiotemporal pattern. Relevance to oscillatory patterns observed in vivo is also discussed.

1570-Pos Board B414
Proteome-Wide Fluctuation Analysis Of S.cerevisiae
Christopher Wood, Joseph Huff, Shiqiang Dai, **Winfried Wiegraebe**.
Stowers Institute for Medical Research, Kansas City, MO, USA.
We measured over 40.000 single live yeast S. cerevisiae cells to determine the concentration and diffusion constants of more than 4100 proteins. These proteins account for more than 75% of the yeast proteome. We used Fluorescence Correlation Spectroscopy (FCS), Photon Counting Histograms (PCH), and Brightness & Number analysis (B&N) to analyze the intensity fluctuations of single molecules fused to GFP. The data was collected using a commercial FCS setup attached to a confocal microscope (ConfoCor3 and LSM 510 META, Carl Zeiss Jena GmbH, Germany) controlled by custom software.
The cells were imaged in transmitted light. We acquired fluorescence images, using the avalanche photo diodes of the FCS setup. This allows us to determine the localization of proteins, the cell cycle as well as cell health.
We developed a software package to automate the measurements and data analysis. We use the Open Microscopy Environment (OME) to organize our images, fluctuation measurements, and analysis results.
We calculated the protein copy number per cell, and compare the noise in concentration levels between different proteins in respect to localization and biochemical pathway.
We find that the diffusion coefficient for GFP is identical in nucleus and cytosol. But interestingly, most of the proteins localized in the nucleus diffuse slower than proteins localized in the cytoplasm.
We will present our data and compare them to information gathered with different methods like flow-cytometry and mass-spectroscopy. We will discuss conclusions derived by complementing our data with information collected in public databases like Saccharomyces Geneome Database (www.yeastgenome.org), Yeast GFP Fusion Localization Database (yeastgfp.ucsf.edu), and the General Repository for Interaction Datasets (www.thebiogrid.org).

1571-Pos Board B415
Macroscopic Singularity In Morphogen Gradient And Bioelectric Field Of Growth Control
Charles Shang.
Harvard Medical School, Boston, MA, USA.
In embryogenesis, every physiological system is developed through a growth control system mediated by organizers and growth control boundaries. The growth control signal transduction is embedded in various physiological functions. Many physiological processes are regulated through growth control mechanisms such as hypertrophy, hyperplasia, atrophy, apoptosis and signal transduction pathways involving growth control genes such as proto-oncogenes. A model of growth control system suggests that a growth control system originates from a network of organizers which distribute at extreme points of structural surface (or interface) curvature. Organizers and growth control boundaries are macroscopic singularities (i.e. discontinuity and abrupt transition) of morphogen gradient field and bioelectric field. Small, nonspecific perturbations around singular points - organizers can have long lasting systemic effect. This offers an efficient way of manipulating the system. The growth control model further suggests that singular points - organizers and separatrices - boundaries in growth control form an undifferentiated, interconnected cellular network that regulates growth and physiology both during and after embryogenesis. Stem cells are important components of this undifferentiated network. Acupuncture points and meridians originate from organizers and growth control boundaries respectively. The model of growth control system has met the gold standard of science - the following predictions of the model have been independently confirmed: 1. Organizers have high electric conductance, high electric current density and high density of gap junctions. 2. Growth control boundaries have high electric conductance and high density of gap junctions. 3. Singularity has important role in morphogenesis. 4. Morphogens and organizers partially retain their regulatory function after embryogenesis. 5. Nonspecific stimulation at acupoints - potential organizers in adult causes extensive growth control effects.
Singularity provides a potentially efficient way of manipulating the growth control, stem cells and physiological systems.

1572-Pos Board B416
Isotopomeric 13C Labeling of Amino Acids Reveal Compartmentation in Saccharomyces uvarum
David W. Schryer[1], Pearu Peterson[1], Toomas Paalme[2], Marko Vendelin[1].
[1]Laboratory of Systems Biology, Institute of Cybernetics at Tallinn University of Technology, Tallinn, Estonia, [2]Department of Food Processing, Tallinn University of Technology, Tallinn, Estonia.
The first step in the synthesis of the aspartic acid (Asp) methionine (Met) and threonine (Thr) can occur in close relatives of *Saccharomyces cerevisiae* such as *S. uvarum* via mitochondrial aspartate aminotransferase (AAT1) or its cytosolic homologue AAT2. Also the amination of cytosolic pyruvate in the production of alanine (Ala) in these species can occur in both compartments using cytosolic (ALT2) or mitochondrial alanine aminotransferase. The aim of this work was to reveal the compartmentation of the synthesis of these amino acids during respiratory growth using isotopomeric data derived from the ^{13}C labeling of proteinogenic amino acids. *S. uvarum* was grown under steady-state growth conditions and fed with a mixture of either $^{13}C[1,2]$ or $^{13}C[2]$ labeled acetate and unlabeled glucose at two dilution rates. Absolute and conditional labeling patterns were measured using ^{13}C NMR and compared with simulated isotopomer distributions within a least squares optimization routine that adjusted the flux parameters. Biomass composition was used to further constrain the fluxes. A software tool was created to automate the composition of the weakly non-linear isotopomer balance equations for all metabolites in the system, thus allowing us to easily test variations of any metabolic network. We properly account for symmetric and prochiral metabolites. The resulting equations are solved without the need for matrix calculations within the optimization routine making this approach a candidate for speeding up the simulation of large metabolic systems. The results of this optimization reaffirm that the precursor for Asp, Met, and Thr is mitochondrial oxaloacetate and that mitochondrial putative alanine transaminase (ALT1) is functional in the synthesis of alanine.

1573-Pos Board B417
Time recoder system of protozoa
Tetsu Saigusa, Atushi Tero, Toshiyuki Nakagaki.
Hokkaido University, Sapporo, Japan.
Unicellular animals might be cleverer than previously thought. Anticipating events are higher functions performed by the brains of higher animals; their evolutionary origins and the way they self- organize, however, remain open questions. Here we show that *Physarum polycephalum* and *Blepharisma japonicum* can anticipate the timing of periodic events. The organisms move rapidly under favourable conditions, but stops moving when transferred to less-favourable conditions. They exposed to low-temperature conditions, presented in several times consecutive pulses at constant intervals, reduced their locomotive speed in response to each episode. When subsequently subjected to favourable conditions, they spontaneously reduced their locomotive speed at the time point when the next unfavourable episode would have occurred. This implied anticipation of impending environmental change. After this behaviour had been evoked several times, the locomotion returned to normal. We explored the mechanisms underlying these behaviours from a dynamical systems perspective. We have developed a dynamical systems model that reproduces the experimentally observed phenomena. Poly-rhythmic amoeboid movement in Physarum has previously been reported by two independent research groups. Oscillations were observed with a series of different periods (600, 240, 30, 10, 2, 0.5, and 0.05 minutes), and the overall activity showed a 1/f-type power spectrum in the Fourier analysis. These results imply that there are oscillations with a series of frequencies and that the frequency distribution is wide and continuous. This is the most fundamental assumption used in the mathematical modeling. Our results hint at the cellular origins of primitive intelligence and imply that simple dynamics might be sucient to explain its emergence.

1574-Pos Board B418
Single-cell Analyses of the *Escherichia Coli* Proteome with Single-molecule Sensitivity
Yuichi Taniguchi[1], Paul J. Choi[1], Huiyi Chen[2], Mohan Babu[3], Andrew Emili[3], Xiaoliang Sunney Xie[1].
[1]Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA, USA, [2]Department of Molecular and Cellular Biology, Harvard University, Cambridge, MA, USA, [3]Donnelly Centre for Cellular and Biomedical Research, University of Toronto, Toronto, ON, Canada.
Genetically identical organisms do not have the same gene expression. This stochastic difference in gene expression, also known as noise, can be the

basis for helping cells cope and survive ever-changing environments. In this work, we profile the heterogeneity of protein expression across the entire Escherichia coli proteome. Our novel approach integrates live-cell single-molecule microscopy with a high-throughput microfluidic platform to systematically reveal noise properties, localization, and functions in the E. coli proteome.

We have constructed chromosomal fluorescent protein fusions for over 1,000 ORFs from the E. coli genome by an efficient, low-cost conversion of an existing Sequential Peptide Affinity tag library. We have developed a microfluidic platform for high-throughput fluorescence microscopy, coupled with automated imaging analysis, enabling us to record the protein expression of over 100,000 cells per hour, which is sufficient to describe the statistics of about 100 different reporter strains. We have measured the distribution of protein expression across cell populations and determined the noise properties of each gene with single molecule sensitivity as necessary. In addition, we have imaged the localization of proteins to the membrane, cytoplasm, and DNA.

To determine possible factors affecting the noise of specific genes, we correlate our protein expression data with biological markers and other global data sets. We find that a substantial fraction of the proteome is expressed at low copy numbers, in agreement with previous predictions, and these genes are subject to high values of noise. We also observe global properties of protein noise in E. coli and find differences in the scaling between noise and average expression for proteins present at low or high copy numbers. Our data provides the first comprehensive proteomic resource of expression levels and noise with high sensitivity for the model organism E. coli.

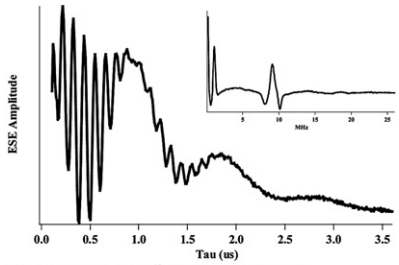
EPR Spectroscopy

1575-Pos Board B419

Multifrequency Pulsed EPR investigation of Fe-histidine Interaction of the Uniquely Coordinated [2Fe-2S] Cluster in the Outer Mitochondrial Membrane Protein, MitoNEET

Michelle M. Dicus[1], Mark L. Paddock[2], Andrea Conlan[3], Patricia A. Jennings[3], Rachel Nechushtai[4], R. David Britt[1].
[1]Dept. of Chemistry, University of California, Davis, Davis, CA, USA, [2]Dept. of Physics, University of California, San Diego, La Jolla, CA, USA, [3]Dept. of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA, USA, [4]Department of Plant and Environmental Sciences, The Wolfson Centre for Applied Structural Biology, The Hebrew University of Jerusalem, Givat Ram, Israel.

Complimentary pulsed EPR techniques (ESEEM, ENDOR, HYSCORE), over multiple frequencies (X, Ka, Q-bands), were used to characterize bonding interactions of the [2Fe-2S] redox active center of the Outer Mitochondrial Membrane protein, MitoNEET. MitoNEET is the first example of a 3Cys-1His coordinated [2Fe-2S] cluster containing protein. Specifically targeting the uniquely single Fe-histidine interaction, EPR investigations integrated both natural abundance ^{14}N and isotopically labeled ^{15}N protein to determine the hyperfine tensor of a strongly coupled imidazole nitrogen of the bound histidine ligand. 1D-ESEEM experiments in the 31, 35GHz frequency region resulted in deep modulation patterns indicative of being near the ''exact cancellation'' limit and was favorable for a more direct spectral assignment of nuclear quadrupolar transition frequencies. Assignment of His87 as the bound ligand was supported by parallel experiments using H87C mutant. An additional advantage of these higher field experiments allows for greater resolved g-anisotropy and a finer degree of orientation-selected experiments, in progress. These should provide a more accurate description of the [2Fe-2S] ligand bonding interaction important for understanding the electronic structure of this new class of redox active proteins.



2-Pulse ESEEM time domain ^{15}N labeled MitoNEET protein (Inset is Fourier Transform-frequency domain) Data taken at CalEPR Center, UC Davis

1576-Pos Board B420

Comparing the Structural Topology and Dynamic Properties of a Model Peripheral Membrane Peptide Magainin-2 Utilizing X- and Q-Band EPR Spectroscopy

Daniel J. Mayo, Nidhi Subbaraman, Johnson J. Inbaraj, Christopher A. Chan, Gary A. Lorigan.
Miami University, Oxford, OH, USA.

Probing the structural and dynamic properties of membrane proteins poses a very difficult problem due to their hydrophobic amino-acid composition and lipid environment they are associated with. To unravel this dilemma lipid membrane mimics have been used to establish a medium by which membrane proteins can be studied. Magnetically aligned phospholipid bilayers (bicelles) coupled with magnetic resonance spectroscopy can be used to extract pertinent information related to their structural topology. This information can be obtained by aligning the samples with respect to the static magnetic field and measuring the corresponding anisotropic spectral parameters. Our lab uses both solid-state NMR and spin-label EPR spectroscopy to study membrane proteins. EPR spectroscopy offers unique advantages over NMR spectroscopy due to a higher sensitivity and a different frequency domain for probing dynamics. These facts have led us to perform EPR spectroscopic alignment studies on the surface peptide magainin-2, which has been shown to exhibit antimicrobial activity by pore formation in two different frequency domains X-Band (9 GHz) and Q-Band (34 GHz). New and unique EPR lineshapes were obtained which not only elegantly contrast integral and peripheral peptide topologies, but also have implications for further elucidating antimicrobial dynamics and their corresponding mechanisms.

1577-Pos Board B421

Crystalline Spin-Labeled Hemoglobin as a Model to Compare Distances Measured by DEER Spectroscopy and X-Ray Crystallography

Zachary M. James, Kurt D. Torgersen, Andrew Thompson, Medora Huseby, David D. Thomas.
University of Minnesota, Minneapolis, MN, USA.

We have recently crystallized and resolved the structure of spin-labeled hemoglobin, while simultaneously using double electron-electron resonance (DEER) spectroscopy to measure inter-spin-label distances within these crystals. Previously, no spin-labeled protein has been analyzed by both DEER-spectroscopy and X-ray crystallography to determine whether the two techniques are in good agreement. Human hemoglobin (Hb) is a useful model system for this comparison, as it readily crystallizes and reacts specifically with the maleimide-TEMPO spin-label (MSL) at Cys93, found within the β subunits of the $\alpha_2\beta_2$ hemoglobin tetramer. For our experiments, we have generated two crystal populations. The first consisted entirely of paramagnetic, EPR-active MSL-Hb, which was used in our X-ray crystallography experiments. The second population contained a low concentration of MSL-Hb in a large excess of hemoglobin labeled with an EPR-silent MSL analog, which assured that distances measured by DEER spectroscopy would not be altered by dipolar interactions between spin-labels of adjacent Hb tetramers. Our results show that both techniques yield similar inter-spin distance measurements, provided that certain precautions are taken to avoid EPR artifacts. We have varied DEER acquisition parameters, such as the dipolar evolution time and acquisition temperature, and analysis methods that affect the accuracy and precision of distance distributions observed by DEER spectroscopy, as compared to those obtained by X-ray crystallography. These results provide the most rigorous analysis to date of the reliability of EPR-based distance measurements. This work was supported by NIH grants (GM27906, AR32961, AG26160, RR22362, GM08700).

1578-Pos Board B422

Structure of the cdb3-ankD34 Complex from Site Directed Spin Labeling Studies

Sunghoon Kim, Eric J. Hustedt, Suzanne Brandon, Charles E. Cobb, Albert H. Beth.
Vanderbilt University, Nashville, TN, USA.

The spectin-based membrane skeleton is responsible for the remarkable mechanical stability and the unique viscoelastisity of the erythrocyte membrane, which are both essential for the survival of red blood cells in the circulatory system. One of the major junctional sites that links the membrane skeleton to the plasma membrane is a protein complex formed by the cytoplasmic domain of band3 (cdb3) and ankyrinR. In this study, site directed spin labeling (SDSL) has been utilized to investigate the global structure of the complex formed between cdb3 and ankD34 (ankyrin repeats 13-24 of full length ankyrinR). We first characterized physicochemical properties of the complex using gel permeation chromatography and sucrose-gradient sedimentation and determined the stoichiometry of the complex to be one cdb3 dimer bound to two ankD34s *in vitro*. For a series of surface sites residing on the binding interface of cdb3, spin label