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Evolutionary Speed of Enzymes Functional Surfaces and their Relationship with Metabolic Fluxes in Networks of Central Carbon Metabolism of Bacteria

David Jimenez-Morales[1], Rudong Li[2], Zhuo Wang[3], Yingzi Li[4], Lei Liu[2], Jie Liang[1].
[1]Bioengineering/Bioinformatics M/C 563, University of Illinois at Chicago, Chicago, IL, USA, [2]The Key Laboratory of Systems Biology, Chinese Academy of Sciences, Shanghai, China, [3]Department of Bioinformatics and Biostatistics, Shanghai Jiaotong U, Shanghai, China, [4]Department of Biomedical Engineering, Shanghai Jiaotong U, Shanghai, China.

Understanding how enzymes emerge, interact, and work together in biochemical networks is a challenging task. The concentrations of enzymes, substrates, products, and their time-dependent changes, the organization of structures of enzymes involved, as well as their evolutionary histories should all be integrated for an improved comprehensive understanding of the systems behavior of the biochemical networks. In this study, we explore the relationship between enzyme structures, enzyme functions, metabolite concentrations and fluxes in the glycolysis and pentose-phosphate pathways, which form the central carbon metabolism of E. coli. As large amount of structural information is available for most components of these metabolic pathways, we have estimated the evolutionary rates of individual functional surface of enzymes in these pathways. To separate selection pressure due to enzyme function from other selection pressures such as structural integrity, folding stability, and folding kinetics, we have estimated the substitution rates of residues located in the functional pockets of these enzyme structures, including residues involved in the catalytic reaction, and residues in the surrounding area participating in the accommodation of both substrates and products. These are then summarized into an overall index of evolutionary speed for enzyme functional surfaces. In conjunction of stoichiometry based flux-balance analysis, we have also simulated time-dependent evolution of metabolite concentrations and fluxes based on ordinary differential equation models and currently available enzyme kinetic parameters. We discuss our findings in overall relationship of evolutionary speed of enzyme function and importance of individual enzyme measured by flux balance and kinetic flow analysis, in the global context of the central carbon metabolism of E coli.

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Functional Assignment of Hypothetical Proteins from Protein-Protein Interaction Networks

Matthias Meier, Doron Gerber, Stephen Quake.
Stanford University, Stanford, CA, USA.

Determining protein functions on proteomic scale is a challenging problem. Almost 8 million non-redundant sequences of proteins are determined from more than 2000 species. The number of protein sequences is doubling every 28 months, however functional assignment for new discovered proteins lacks behind. Computational methods based on sequence or structure similarities, clustering of co-regulated genes, and phylogenetic profile mapping are reliable for about eighty percent of all protein sequences. For the remaining twenty percent the functional assignment fails. Elucidation of protein function and localization in the cell for uncharacterized proteins will be inevitable to describe biological processes on a systems level. Here we present a high-throughput experimental method to assign protein function of uncharacterized proteins based on protein-protein interaction networks. We demonstrate that it is possible to generate a whole genome protein-protein interaction map for one protein in a day experiment. For this we exploit a recently developed microfluidic chip. The highly integrated device is a combination of rapid prototyping in polydimethylsiloxane and DNA microarray technology. One microfluidic device allows in vitro expression of thousands of proteins and can perform 4K high affinity measurements in parallel. In order to be able to characterize unknown proteins by protein-protein interactions on a proteomic scale we constructed a cDNA library of the Streptoccocus pneumonia (SP) proteome. After optimization 2/3 of the SP proteome could be expressed on chip and used for high throughput screening. For functional assignment of 25 highly conserved hypothetical or hypothetical proteins specific for the SP organism we performed 35,000 affinity measurements. The resulting interaction network and its network parameters are used to categorize the hypothetical proteins to common functional classes.

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Hydration Pressure Increases in Heat-Damaged Skin:osmotic-Stress and High-Resolution Magnetic Resonance Data Implicate Local Supramolecular Mechanisms

Maria P. McGee, Michael Morykwas, Louis Argenta.
Wake-Forest University Medical School, Winston-salem, NC, USA.

Local forces driving interstitial-fluid transfer affect burns; heat-ablation and remodeling therapies; hemodynamics; and wound healing. We examined hydration/dehydration mechanisms after heating skin ex vivo at 40, 50, and 60°C to induce ''denaturing'' transitions in three main structures of the interstitial matrix: hyaluronic acid, cells, and collagen.

Hydration pressure (HP), swelling rates, water activity, acid-binding capacity (at pH~7.4) and transverse nuclear-relaxation rate of solvent-water protons (1/T2) were compared using osmotic-stress, gravidometric, saturation vapor-pressure, potentiometric, and magnetic resonance imaging (μMRI, 40μm /pixel resolution) analyses, respectively. Mean HP values were 89, 100, 79, 108 at 4°C and 50, 62, 91, 162 mmHg at 37°C in nonheated control, 40, 50, and 60°C samples, respectively. Swelling kinetic energy increased 1.1, 4.5, and 6.9-fold relative to controls. Acid-binding and water activity increased by 0.3 and 0.4 pH units and 0.020 and 0.033 (at 75% hydration) in 50 and 60°C samples, while in 40°C samples, values remained close to controls'. μMRI changes were obvious only in 60°C samples with 1/T2 gradients (from T2-map contrast), and average 1/T2 in the central dermis decreased 0.1 intensity units/μm and 66%, respectively.

HP and acid-binding data indicate increases in the total solvent-exposed area and, with mutually consistent 1/T2 decreases and water-activity increases, demonstrate qualitative changes in its average hydration properties. Quantitative differences among control and preheated samples and between hydration parameters obtained at 4 versus 37°C confirm temperature-dependent supramolecular reorganization following damage. Together, results provide strong evidence that multiscale interactions among matrix components, independent of vascular, hormonal and neural controls, change key interstitium fluid-transfer properties with heat-damage.

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Reactivity of Human Holocarboxylase Synthetase Toward Biotin-Accepting Substrates

Maria Ingaramo, Dorothy Beckett.
University of Maryland, College Park, MD, USA.

Human Holocarboxylase Synthetase (HCS) catalyzes biotin transfer to carboxylases in a two-step reaction in which the activated biotin, bio-5′-AMP, is first formed from substrates biotin and ATP. The biotin is then transferred to a single lysine residue on the carboxylase. The five substrates for HCS, including acetyl-CoA carboxylases 1 and 2, pyruvate carboxylase, 3-methylcrotonyl-CoA carboxylase and propionyl-CoA carboxylase, play critical roles in metabolism. Two isoforms of HCS differ at the amino-terminus by 57 amino acids, and their specificity towards the substrates is unknown. The biochemistry of HCS function has been investigated by characterizing the basic properties of the two isoforms and their interactions with the five acceptor-protein substrates. Equilibrium sedimentation indicates that the proteins are monomers in their apo-forms and when bound to the adenylated intermediate. Steady state analysis of the overall reaction indicates that both isoforms possess similar behavior with respect to the small molecule substrates. In contrast, stopped flow fluorescence measurements of biotin transfer indicate that the HCS forms can exhibit distinct association rates with a single biotin acceptor. Moreover, the isoforms display preferential reactivity among the substrates. These results are consistent with a role for HCS N-terminus in acceptor substrate recognition, and suggest a role for HCS in dictating a hierarchy of biotin utilization by carboxylases.

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Biological Failure Model for Pancreatic β-Cell Dysfunctions

Hyuk Kang[1], Kyungreem Han[2], Jinwoong Kim[2], Moo Young Choi[3].
[1]Korea Institute for Advanced Study, Seoul, Republic of Korea, [2]College of Pharmacy, Seoul National University, Seoul, Republic of Korea, [3]Department of Physics and Center for Theoretical Physics, Seoul National University, Seoul, Republic of Korea.

Dysfunctions of pancreatic β-cells are one of the most critical features for both T1DM and T2DM. Although autoimmune attack is thought to be most common cause of T1DM, the detailed mechanisms of the progression from mild pancreatitis to severe β-cell dysfunctions are not fully understood. On

the other hand, in the case of T2DM, chronic glucose and lipid over accumulations cause β-cell dysfunctions. The physiological parameters used in clinics such as plasma insulin concentrations, however, seem to be insufficient for the understanding the dynamics of each β-cells. In numerous cases, during many years of subclinical periods, they don't have insulin deficiency on their early stages. For the universal understanding of such complex backgrounds, we apply a simple physical model. The model consists of L^2 lattice array of β-cells which are electrically coupled with each other and they interact with brain, liver, muscles and fats. We consider the blood glucose level as an external stress and assume that each of β-cells has its own threshold of damage. For the given blood glucose level, the β-cells with low-threshold are damaged more easily. As the extra loads of the dysfunctional β-cells are transferred to the normal β-cells, they are damaged more easily. Considering the load sharing, we numerically simulate overall β-cell dysfunction as different normal β-cell numbers. Regarding the activities of K_{ATP} channel as the β-cell function, we obtained increasing patterns of the blood glucose level in the body and the decreasing patterns of β-cell membrane bursting and insulin secretions of each β-cells.

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Noise Can Induce Steady-State Bimodality in Positive Feedback Loops Without Cooperativity

Tsz-Leung To, Narendra Maheshri.
MIT, Cambridge, MA, USA.

Positive feedback is a common network motif in gene regulatory networks that is widely recognized to lead to bistability and, as a consequence, to hysteresis and switch-like responses. A non-linear, cooperative promoter response provides the necessary ingredient to generate bistability in deterministic descriptions of positive feedback. Using a synthetic system, we show experimentally that positive feedback is capable of inducing a bimodal, switch-like response with non-cooperative feedback, even when the underlying deterministic dynamics do not admit bistability. In accordance with theoretical models, the bimodal response requires the promoter within the feedback loop to be noisy, with infrequent, large bursts of expression. In addition, the transcription factor (TF) involved in the feedback loop has to be short-lived. Using a stochastic model and experimentally measured in vivo parameters of the promoter response in the absence of feedback, we can quantitatively describe the feedback response. We also find that multiple TF binding sites in a promoter can be important for the bimodal response not because of molecular cooperativity in TF binding, but because of increased noise in the promoter. Because many promoters possess multiple binding sites and many TFs are unstable, positive feedback loops in many gene regulatory networks may exhibit bimodal responses, but not necessarily because of deterministic bistability as is commonly thought.

Vibrational Spectroscopy

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Micro-Spectroscopy of Biomolecules and Cells at Variable Pressure in a Micro-Capillary

Sang Hoon Park, Silki Arora, **Alfons Schulte**.
University of Central Florida, Orlando, FL, USA.

Combining Raman microscopy with a micro-capillary compartment enables spectroscopic studies of small amounts of biological material at variable pressure. We present experiments over the pressure range from atmospheric pressure to 4 kBar in a micro-capillary that use less than 100 nanoliters of sample. We investigate pressure dependent structural changes in DMPC and POPC lipid systems through measurements of the Raman spectrum in the CH2 stretch band and fingerprint regions. The micro-capillary also allows to enclose living cells and to optically interrogate them through a microscope. This is illustrated through Raman spectroscopy and direct optical imaging of individual red blood cells at variable pressure.

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Raman Spectroscopic Signatures of the Metabolic States of Escherichia Coli Cells and their Dependence on Antibiotics Treatment

Tobias J. Moritz[1], Douglas S. Taylor[2], Christopher R. Polage[2], Denise M. Krol[3], Stephen M. Lane[1], James W. Chan[1].
[1]NSF Center for Biophotonics Science and Technology, UC Davis, Sacramento, CA, USA, [2]UC Davis Medical Center, Sacramento, CA, USA, [3]UC Davis, Davis, CA, USA.

The metabolic states of Escherichia coli (E. coli) cells were characterized with laser tweezers Raman spectroscopy and the cellular response to the antibiotics Cefazolin and Penicillin/Streptomycin were correlated to spectroscopic changes.
The Raman spectra of *E. coli* cells were measured at different time points in the bacterial growth curve, which revealed several spectral features applicable for identification of the bacterial cell's growth phase. The time dependent behavior of Raman peak intensities allowed us to identify four groups of Raman peaks with similar intensity time trends, displaying specificity to the same bio-molecule. The intensity of Raman peaks associated with DNA increased over time in contrast to protein specific Raman peaks, which decreased at different rates. In addition, the intensity of the adenine ring-breathing mode increased initially and decreased after approximately 10h. A separation of Raman spectra specific to different metabolic states of E. coli cells was visualized with principal component analysis (PCA).
The exposure of bacterial cells to Cefazolin and Penicillin/Streptomycin at the end of the exponential growth phase, resulted in differences in the time dependence of several Raman intensities. In addition, a shift of the spectral position of the adenine ring-breathing mode, associated with normal growth, was not observed for either of the two antibiotic drug exposures. The time dependence of the amide I Raman band intensity changed for Penicillin/Streptomycin exposure compared to normal growth, but not for Cefazolin treatment. This suggests that Raman spectroscopic markers of cellular response may differ between antibiotics or drug classes.

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Data Processing in FTIR Imaging of Cells and Tissues: Towards Protein Secondary Structure Imaging

Erik Goormaghtigh, Audrey Bénard, Allison Derenne, Régis Gasper.
Université Libre de Bruxelles, Brussels, Belgium.

IR spectroscopic images were recorded using an Equinox Bruker FTIR spectrometer coupled to a Hyperion 3000 imaging system equipped with a mercury cadmium telluride (MCT)-based focal plane array (FPA) detector of 64×64 pixels (Bruker Optik, Ettlingen, Germany). Images containing 4096 IR spectra at 8 cm-1 spectral resolution were acquired by coadding 256 interferograms in about 5 minutes. All the data processing was carried out by the program ''Kinetics'' running under MatLab. The ''Kinetics'' software, previously used for FTIR spectrum processing and analysis, was extended for the processing of FPA-acquired FTIR data. Different types of images can be generated, either based on the absorbance at 1 wavenumber or the ratio of the absorbances at 2 wavenumbers or more advanced combination of the data. We show here that protein secondary structure content evaluated as described in [1] sheds light on the molecular determinants that allow the differentiation between sub-structures in tissues. Similarly we shall illustrate heterogeneity in prostate cancer cells (PC-3) in culture based on secondary structure imaging.
References
[1] Goormaghtigh,E., J.M.Ruysschaert, and V.Raussens. Evaluation of the information content in infrared spectra for protein secondary structure determination, Biophys.J. 90 (2006) 2946-2957.

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Inter-Residue Coupling of Model PPII Helices using 13c Isotopic Labeling

heng chi, Ahmed Lakhani, Anjan Roy, Timothy A. Keiderling.
UIC, chicago, IL, USA.

Characterization of poly-proline II (PPII) conformation on a site-specific basis has importance in developing a model for structure and stability in these systems. Coupling of selected residues for a series of related peptides having predominantly PPII conformations were measured using VCD and IR spectra of selected variants that were doubly labeled with ^{13}C on the amide C=O. The characteristics of the ^{13}C=O component of the amide I'IR band and their sensitivity to the local structure of the peptide are compared to predictions based on DFT level calculations for related structures and used to determine coupling between C=O groups along the backbone of this helical structure. Doubly labeled peptides have spectral shifts reflecting the mass change in addition to coupling between residues. In the PPII case the coupling is relatively weak, yet by combining IR and VCD along with DFT level calculations, we have been able to determine its coupling constants. Comparison of PPII structures with ''random coils'' can be done by comparing all Proline, mixed Ala-Pro and Lys-rich sequences. The shifts and couplings reflect the computations in all cases. The distinct vibrational coupling patterns of the labeled sites based on this structure are also well matched by *ab initio* DFT-level calculations of their IR and VCD spectral patterns.