been conducted at a range of temperatures that affect the binding and catalytic activities. Our findings challenge traditional assumptions about the role of long-range surface diffusion and offer a glimpse on the processivity of cellulases.

3893-Pos
Investigation of the Effects of Cellulose Morphology on Synergism in Cellulase Mixtures using Quantitative Fluorescence Microscopy
Marie K. Donnelly, Jose M. Moran-Mirabal, Stephane C. Corgie, Harold G. Craighead, Larry P. Walker.
Cornell University, Ithaca, NY, USA.
Efficient and economic conversion of plant cell-wall materials into fermentable sugars is the principal technical challenge in making biofuels an important part of our energy future. At the most fundamental scale, cellulose hydrolysis occurs when cellulases bind to cellulose polymers and hydrolyze the β-1,4 linkages between glucose monomers. However, gaining access to polymers requires that cellulases diffuse and bind to complex three dimensional structures such as cellulose microfibers, mats, and particles. In addition, cellulases exhibit different binding characteristics depending on their reactive domains. Though individual cellulases hydrolyze cellulose relatively slowly, mixtures of cellulases and other plant cell wall degrading enzymes act synergistically to enhance rates and extents of hydrolysis. Elucidating the molecular mechanisms that give rise to synergistic behavior is an important research goal. A major question is to what degree does cellulose morphological structure - particle shape, size and pore structure - influence accessibility to cellulose polymers and how this accessibility changes with different cellulases.
To investigate the effects of cellulose macrostructure on synergism, pure fluorescently labeled populations of cellulases Cel5A, an endocellulase, and Cel6B, an exocellulase, were applied to cellulose immobilized in the form of mats, bundles and isolated cellulose fibrils. Cellulases were labeled with one of two different fluorophores and purified for a known degree of labeling. Cellulase binding on cellulose was recorded using time-lapsed fluorescence microscopy with images taken at defined time intervals. Local cellulase concentration on the cellulose fibrils was estimated using a standard curve obtained from known fluorophore concentrations. Binding kinetics curves were established for mixtures of these two cellulases to observe the time scale of cellulase diffusion and to observe how diffusion determines what fraction of each cellulase can occupy the system space to react synergistically.

3894-Pos
Live Cell Fluorescence Analysis and Mathematical Modeling of Dual Mechanisms of Cdc42 Recycling in Yeast: Relationship of Internalization Rate and Morphology
Brian D. Slaughter[1], Arupratan Das[1], Boris Rubinstein[1], Jay Unruh[1], Joel W. Schwartz[2], Rong Li[1,3].
[1]Stowers Institute for Medical Research, Kansas City, MO, USA, [2]Duke University, Durham, NC, USA, [3]Department of Molecular and Integrative Physiology, Univerity of Kansas Medical Center, Kansas City, KS, USA.
Maintenance of robust cell polarization is a pre-requisite for oriented growth or motility biological systems. Cdc42 is a Rho-GTPase that, once polarized, organizes the deposition of growth materials to the polarized site. Previous studies in yeast have found that after initial polarity establishment, the individual molecules of Cdc42 are rapidly exchanged between the membrane and the cytosol. Despite these rapid dynamics, the site of Cdc42 accumulation is somehow held rigid to allow for polarized growth. We show that after initial symmetry breaking, Cdc42 is dynamically maintained at the polar cap through two recycling mechanisms: actin-mediated transport / endocytosis and cytosolic recycling through the Rho-GDP dissociation inhibitor (GDI) Rdi1. However, the mechanism by which the spatial relationship of these dual recycling pathways is controlled is unclear. We combined detailed live cell fluorescence measurements, including FRAP, iFRAP and fluorescence cross-correlation spectroscopy, with a thorough mathematical model to examine how dual recycling pathways of Cdc42 in yeast work together to shape the Cdc42 membrane distribution. We find that in order to recapitulate the steady-state membrane distribution of WT Cdc42, the dual recycling pathways must employ overlapping delivery windows of similar size. Interestingly, the modeling of live cell fluorescence data reveals that Rdi1-mediated tuning of a single dynamic parameter, internalization rate inside the delivery window, is sufficient to explain differences in the Cdc42 cap distribution between yeast cells destined for different morphogenic fates. These changes in Cdc42 cap distribution are correlated to the observed morphogenic differences under these conditions.

3895-Pos
Self-Referencing Differential Spectroscopy Analysis in Breast Translational Research
Shanshan Xu, Albert Cerussi, Enrico Gratton.
University of California, Irvine, Irvine, CA, USA.
A self-referencing differential spectroscopy analysis approach has been developed for broadband near-infrared (NIR 650-1000 nm) absorption spectra to reveal intrinsic optical breast cancer biomarkers. Through the application of this method that accounts for inter-patient variability using the normal tissue as an internal control, we have characterized the metabolic differences between malignant and normal tissues that result from subtle alterations in molecular disposition.
From a pilot study of 15 cancer patients performed in 2007, absorption signatures, not arising from the individual abundance in the four major chromophores (lipid, oxy-hemoglobin, deoxy-hemoglobin and water), have been demonstrated to successfully differentiate the normal and malignant tissues. Based on the data acquired from a NIR Diffuse Optical Spectroscopy Imaging instrument, specific spectral signatures containing specific NIR absorption bands are located in regions at about 760, 930, and 980 nm indicative of lipid biomarkers or water in abnormal state. The shape of the fingerprint spectra, namely specific tumor component (STC) spectra, is highly reproducible and exhibits consistent and particular wavelength-dependent characteristics. STC index algorithm was set up to quantitatively computing the residual due to components that are unaccounted for by the basis spectra.
A 61 subject retrospective study aiming to distinguish between benign and malignant breast tumors was carried out in 2008 on top of previous findings. By converting the observed molecular dispositions into a simple index (malignancy index) derived from a weighted wavelength analysis to maximize the differences between the benign and malignant tumors, two types of tumors were stratified with 95% sensitivity, 89% specificity, 91% positive predictive value, and 94% negative predictive value. The observation of pathology specific spectral signatures provides a potentially substantial method for differential diagnosis and monitoring response to neoadjuvant chemotherapy or hormonal therapy.
Work supported in part by NIH-P41 P41-RRO3155 and P50-GM076516

3896-Pos
Determination of Regional Diffusion Coefficients of Fluorescent ATP in Rat Cardiomyocytes
Ardo Illaste, Martin Laasmaa, David Schryer, Rikke Birkedal, Pearu Peterson, Marko Vendelin.
Tallinn University of Technology, Tallinn, Estonia.
Results from several experiments indicate the existence of restrictions to diffusion of ADP/ATP in permeabilized rat cardiomyocytes. Decreased rate of diffusion is necessary to explain measurements of kinetics of respiration, sarcoplasmic reticulum loading with calcium, and kinetics of ATP-sensitive potassium channels. Determining intracellular diffusion coefficients allows for estimation of the effect of these restrictions on bioenergetics. Previously we extended raster image correlation spectroscopy (RICS) methods and demonstrated the existence of anisotropy in the diffusion of fluorescently labeled ATP in rat cardiomyocytes. Specifically, longitudinal and transverse diffusion coefficient was, respectively, ~2 and ~3.5 times smaller than in solution. We attempted to extend our method in order to construct regional maps of diffusion coefficients. Our efforts using a commercial confocal microscope failed due to shortcomings in protocol automation, photon detection and noise properties at high scanning speeds. In order to solve these issues, we custom built a confocal microscope and wrote software to automate the protocol for performing RICS measurements with varying scan speeds and rotation angles. During calibration, we demonstrated that the direction of the scan can influence autocorrelation function calculated according to RICS protocols through non-symmetric point spread function. With the new system, we have been able to measure regional diffusion coefficients in permeabilized rat cardiomyocytes. As expected, there is a sharp change of the diffusion coefficient on the border of the permeabilized cell. Interestingly, the values of diffusion coefficients estimated for the regions surrounding the cell are similar to the ones determined in plain solution. While there has been some variation in diffusion coefficients within the cell, the present precision of the method does not allow us to distinguish the diffusion coefficients in regions smaller than few micrometers. For that, further development and experiments are needed.