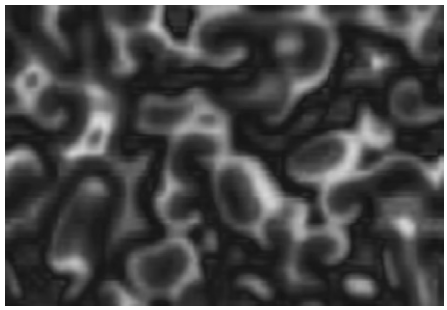

the proper continuum limit - is aptly captured by a set of stochastic partial differential equations. The system's stochastic dynamics is shown to lead to the emergence of entangled rotating spiral waves. While the spirals' wavelength and spreading velocity is demonstrated to be accurately predicted by a (deterministic) complex Ginzburg-Landau equation, their entanglement results from the inherent stochastic nature of the system. [Nature 448, 1046-1049 (2007)]

Biotechnology & Bioengineering II

3259-Pos Board B306

Prototype and Applications for Asynchronous Rotation of Magnetic Microspheres

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Magnetic microsphere suspended in a fluid aligns its magnetic moment with external magnetic field and follows a rotating external field with a constant phase lag. While this is true for low enough driving frequencies, the dynamics of the rotation change above some critical frequency and the particle rotates asynchronously with the driving field. This nonlinear response of the microsphere depends on physical parameters such as the magnetic moment and the size of the particle as well as the viscosity of the surrounding fluid.

Asynchronous rotation of magnetic microspheres has many applications including magnetic particle characterization, viscosity measurements in small amounts of fluid, and pathogen detection. The technique enables continuous sensing of the sample which allows for real time viscosity measurements and single cell growth analysis.

One of our technological and research goals is to develop a portable, easy to use and low power device that utilizes a magnetic field to asynchronously rotate magnetic particles. Owing to the platform technology nature of the method, the prototype setup explained in this poster can be utilized in many applications with minor modifications. Asynchronous rotation analysis can be done using off the shelf magnetic particles (usually used for magnetic separation) or custom made Janus particles (MagMoons) depending on the application. This poster will discuss progress toward this device as well as the applications of asynchronous rotation of magnetic microspheres.

3260-Pos Board B307

Unnatural Amino Acid Mutagenesis For Site-specific Incorporation Of Keto And Azido Functionalities Into Functional G Protein-coupled Receptors

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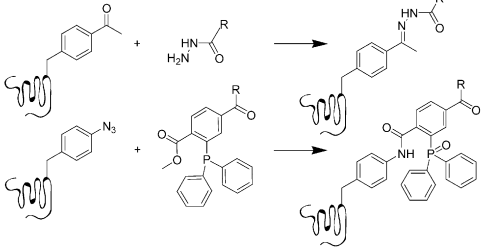
The insertion of unnatural amino acids into proteins using amber stop codon suppression has shown promise as a technique for probing protein structures. To investigate applications to studies of G protein-coupled receptors, we have developed methods that allow incorporation of each of three tyrosine analogues – *p*-acetyl-phenylalanine (Acp), *p*-benzoyl-phenylalanine (Bzp) (Ye, Kohrer et al. 2008), and *p*-azido-phenylalanine (Azp) - into GPCRs site-specifically at high yields in mammalian cell culture. The unique keto and azido functionalities allow specific attachment of tags and fluorophores into GPCRs by hydrazone and Staudinger-Bertozzi ligation respectively under physiological conditions. Together with cysteine-specific labeling methods, our technique will make it possible to introduce pairs of fluorophores in a general way. This is a prerequisite for single molecule fluorescent resonance energy transfer (smFRET) studies, which will yield receptor dynamic information not readily available by other experimental methods.



3261-Pos Board B308

pHLIP-bionanosyringe for Targeting Acidic Solid Tumors and Selective Delivery of Nanomaterials

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We have found a way to target tumors based on their elevated levels of extracellular acidity. Acidosis is a hallmark of tumor development both at very early and at advanced stages. However, the acidic extracellular environment in tumors has not been properly explored yet probably due to a lack of compounds that dramatically change their properties in the range of pH 6.0-7.5. Recently we designed the pH Low Insertion Peptide (pHLIP), which acts as a bionanosyringe, it inserts into cellular membrane and forms transmembrane helix at acidic extracellular pH (6.0-6.5) but not at normal pH. Our data demonstrated that the fluorescently labeled pHLIP was accumulated in tumors established in mice. pHLIP can find cancer cells and insert itself in cell membranes. No insertion occurs in normal cells (pH 7.4). The pHLIP can be used to deliver various compounds, including diagnostic probes, drugs, nanomaterials, radiation or photosensitizers and thermosensitizers, to or into cancer cells. Here we demonstrated that pHLIP can selectively deliver near-red dyes, gold nanoparticles and carbon nanotubes to the tumors established in mice. We found that pHLIP targeted particularly well on the highly metastatic tumors including newly formed metastatic lesions. Our technology opens the new opportunity to target cancer tumors with high selectivity and decrease side effects. The work has been supported by grants from the Department of Defense PCRP CDMRP BC061356 and National Institutes of Health NCI133890.

3262-Pos Board B309

Controlled Assembly of Vesicle Layers on Layer-by-layer Particles via DNA Hybridization

Martin Loew[1], Jing Kang[2], Lars Dähne[2], Oliver Kaczmarek[1], Jürgen Liebscher[1], Daniel Huster[3], Kai Ludwig[4], Christoph Böttcher[4], Andreas Herrmann[1], Anna Arbuzova[1].
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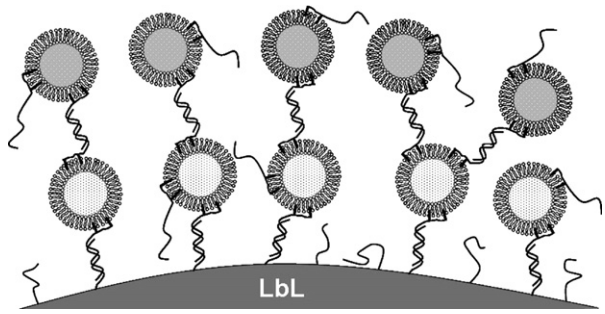
We report here on the formation of layers of large unilamellar vesicles (LUVs) superimposed on Layer-by-Layer- (LbL-) particles. DNA oligonucleotides were covalently attached to the outermost negatively charged polyelectrolyte layer of the particles and thus vesicles, with complementary lipophilic DNAs incorporated into the membranes, could be assembled in layers via sequence specific hybridization (see figure). Entrapment of calcein, NBD-rhodamine FRET fusion assay, FRAP, and cryo electron microscopy proved that LUVs attached to LbL-particles remained intact. The assembly was reversible, e.g. heating above the melting temperature of the DNA-hybrids led to the dissociation of the vesicle layer. Fusion of vesicles attached to the LbL-particles and leakage of the entrapped molecules was triggered on demand by addition of melittin. Using different DNA sequences, lipid anchors or compositions of the membrane can regulate the assembly of layers. The LUVs-LbL-particles have many advantages: a controlled and reversible assembly, small and defined size, easy manipulation, biocompatibility, and biodegradability of the particles, and the possibility of a triggered release of different reactants entrapped in different layers of vesicles. LUVs-LbL-particles can be potentially used in diagnostics or for the organization and regulation of reactions on nanoscale.



3263-Pos Board B310

Measurement Of Hydrogen Ion Activity In The Intercellular Space Of Schwannoma Tumors

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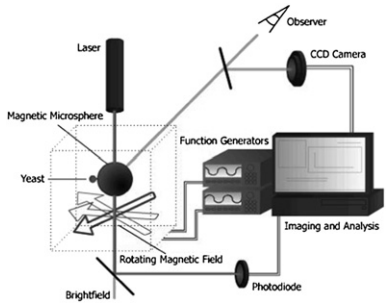
We are exploring the use extracellular fluorescent indicators to measure analytes in the microdomains between adjacent cells in tissues. Our primary initial goal is to measure changes in H^+ activity in the intercellular space of xenograft tumor models before and after drug treatment. It is thought that acidification in the core of tumors protects these cancers from the primarily, weak base, chemotherapeutic drugs. We are comparing the use of functionalized nanoparticles and expressible indicators as putative extracellular sensors. Functionalized, fluorescein-labeled nanoparticles were constructed that enable the particles to adhere to the surface of schwannoma cells. However, preliminary work with the nanoparticles showed toxic side effects, at least over a period of days. We have also been exploring the use of expressible membrane bound H^+ indicators, glycosylphosphatidylinositol (GPI)-anchored GFP mutants. A problem with these indicators is that they localize to the plasma membrane and also the entire exocytotic pathway. *In vitro* testing of the indicators was accomplished by aggregating single cells into clusters (artificial tumors) using dielectrophoresis (DEP) and agarose molds. Preliminary results indicated that nanoparticles could be trapped within artificial tumors using DEP as long as electric fields are applied. Long term (7 days) intercellular pH sensing was achieved *in vitro* using GPI-GFP expressing schwannoma cells in an artificial tumor crafted with an agarose mold. While we are currently exploring the use of sensors to measure changes in pH in the intercellular space, we are also investigating the development and testing of other sensors to detect analytes such as ATP, Cl^-, Na^+, and K^+. This will provide valuable information regarding cellular defenses against chemotherapeutic drugs and mechanisms of therapeutic drug action.
This project was funded by the NIH:NCRR as a national research resource [P41 RR001395]

3264-Pos Board B311
Using Automated Cell Tracking Software to Quantifying Durokinesis and Durotaxis in Real Time
Matthew L. Walker, David House, Margrit Betke, Joyce Y. Wong.
Boston University, Boston, MA, USA.
In vivo, there is an intimate connection between certain cellular processes and the physical nature of the surrounding environment. Specifically, it has been theorized that changes in the physical properties of the extra-cellular environment within the vasculature influence cellular migration which can influence such processes as angiogenesis and occlusive vascular disease.
In order to observe and quantify the compliance directed migration of both vascular smooth muscle cells and fibroblasts, we employed both polyacrylamide substrates, in which the tensile modulus could be tuned to specific values, and novel computer imaging software which automatically tracked cellular movement in less then ideal optical imaging conditions.
Although both durotaxis and durokinesis have been previously observed in large population studies, our application of computer vision software allowed for a high throughput analysis of individual cells in real time. This method not only standardized the data collection but also enabled us to observe and quantify changes in speed, angular deviation, acceleration and deceleration within a single cell's migration track as a function of substrate stiffness and in the presences of a compliance gradient. This detailed analysis will serve to refine our understanding of cells respond to the physical stimuli presented in the environment.

3265-Pos Board B312
Asynchronous Rotation as a Rapid and Sensitive Technique for Quantifying Cell Growth Dynamics
Daniel W. Youngstrom, Brandon McNaughton, Päivö Kinnunen, Raoul Kopelman.
University of Michigan, Ann Arbor, MI, USA.
The ability to monitor physical parameters of cell growth dynamics on an increasingly sensitive scale is of great interest. Nonlinear rotation of cell-coated magnetic microspheres is an exciting new technique for rapid cell detection and measurement. Previous explorations of this approach primarily involved bacteria and other prokaryotes, but new methods demonstrate it is possible to extend this model to the world of eukaryotes, specifically simple yeasts. In this experiment, *Saccharomyces cerevisiae* cells were coated in biotin and covalently linked to a streptavidin-coated magnetic microsphere. With their cell membranes bound to the sphere, the unit was rotated asynchronously in a magnetic field. As the cells grow, the viscous drag experienced by the cell-bearing microsphere increases, counteracting the magnetic driving force, yielding a steady overall increase in rotational period. The rotation rate is actively monitored by specialized computer software using the voltage output of a laser and photodiode.



Current data reinforces the success of dynamic asynchronous rotation as a valid means for rapid and sensitive growth detection. In the future it is believed this technique may be further extended to the study of increasingly complex organisms, including mammalian cells.

3266-Pos Board B313
Detection Of Drinks Contamination Using Optical Refractometry Technique (ORT)
Christopher E. Bassey, Cynthia A. Siguenza.
Azusa Pacific University, Azusa, CA, USA.
Optical Refractometry Technique (ORT) has been used to characterize unknown substances, check the concentration of known substances, and also determine the sugar content of a given liquid. It involves the determination of a medium's refractive index, which is a measure of the speed of light in the medium. The technique is based on the principle that the speed of light in a given medium is a reflection of its absorption and emission characteristics. The speed of light also depends on the physical state, composition, and molecular structure of the medium. By comparing the optical properties of pure drinks with those of drinks tainted with foreign agents, the level of contamination can be detected. This work examines the feasibility of using ORT to detect the contamination Gatorade drink with antifreeze, which has already led to a number of deaths. The results will enhance the development of instrumentation and methodology for continuous monitoring and detection of possible contamination.

3267-Pos Board B314
Assessment of Cytotoxicity by Analysis of Impedance Fluctuations
Jun-Chih Lo, Daniel Opp, Chun-Min Lo.
University of South Florida, Tampa, FL, USA.
Electric cell-substrate impedance sensing (ECIS) has been used to monitor cell behavior in tissue culture and has proven to be very sensitive to cell morphological changes and cell motility. In this method, cells are cultured on small gold electrodes carrying weak AC signals. The impedance of these electrodes changes dramatically when cells attach and spread on their surface, because the cell membranes restrict the current flow. In addition, cell motion may reveal itself as a fluctuation in the measured impedance, which is always associated with living cells and persists even when the cells grow into a confluent layer. The impedance fluctuation is attributed to incessant changes in the size of the cell-substrate space as cells persistently rearrange their cell-substrate adhesion sites. The magnitude of this sort of vertical motion detected by ECIS is of the order of nanometers and referred to as micromotion. Here, we applied ECIS to evaluate dose-dependent responses of NIH 3T3 cells exposed to cytochalasin B, cadmium chloride, and H-7 dihydrochloride, a protein kinase C inhibitor. To detect the alternation of cell micromotion in response to cytotoxic challenge, time-series impedance fluctuations of cell-covered electrodes were monitored and the values of power spectrum, variance, and variance of the increment were calculated to verify the difference. While a dose-dependent relationship for each chemical was generally observed from the overall resistance of the cell monolayer, the analysis of impedance fluctuations distinguished cytochalasin B, cadmium chloride, and H-7 dihydrochloride levels as low as 0.1, 10, and 1 micromole respectively. The analytical methods used in this study can serve as a model approach for ECIS and other electrochemical impedance biosensors to investigate various aspects of cellular responses to toxins in general.

3268-Pos Board B315
Towards In Silico Bioprinting
Adrian Neagu[1,2], Elijah Flenner[1,3], Bogdan Barz[1], Cyrille Norotte[1], Francoise Marga[1], Karoly Jakab[4], Ioan Kosztin[1], Gabor Forgacs[1].
[1]University of Missouri-Columbia, Columbia, MO, USA, [2]Victor Babes University of Medicine and Pharmacy, Timisoara, Romania, [3]Colorado State University, Fort Collins, CO, USA, [4]University of Virginia, Charlottesville, VA, USA.
Bioprinting is a computer-controlled procedure for building three-dimensional tissue constructs via layer-by-layer delivery of cells and supportive hydrogels. To describe the post-printing self-assembly of multicellular structures, we performed computer simulations that incorporate a basic principle of developmental biology, the differential adhesion hypothesis (DAH). DAH states that cell motility combined with differences in the adhesive properties of different cell types yields tissue conformations with the largest number of strong bonds