enough to represent 67% of the variance in cell area in *wt, mlcE-* and *mhcA-* cells. The three principal shape modes are dilation/elongation, a half-moon shape and bulging of the front/back. The second of these modes represents sideways protrusion/retraction, is associated to lateral asymmetries in the cell traction forces / F-actin distribution, and is significantly less important in *mhcA-* cells. These results indicate that the mechanical cycle of traction stresses and cell shape remains similar but is slowed down when myosin function is lost, probably due to a reduced control on the spatial organization of the traction stresses.

3253-Pos Board B300

Bihelical waves: A novel form of eukaryotic cell motility exhibited by African trypanosomes

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Flagella and cilia play a critical role in eukaryotic cell motility. Among the most notable waveforms exhibited by eukaryotic flagella are planar and helical waves observed in mammalian sperm and protozoa. Here we report on a high-speed study of the flagellar motility of the protozoan parasite Trypanosoma brucei responsible for the African sleeping sickness whose vector is the tsetse fly. In this organism, the flagellum is physically attached along the length of the tapering cell body, unlike the case of mammalian sperm where the flagellum is attached to the body only at one attachment site. Earlier studies had reported that propulsion was driven by helical waves propagating from the flagellar tip to the base with left-handed helicity. Using a millisecond-timescale microscope, we discovered a novel form of eukaryotic cell motility, in which alternating left-handed and right-handed helical waves (termed ''bihelical waves'') propagate along the flagellum and are separated by a moving kink. These bihelical waves produce torsion in the cell body that is resolved by a rocking motion but - unlike the case of mammalian sperm or the existing model for T. brucei - without net rotation. We also observed the rapid motion of the flagellum tip, for which we recorded velocities up to 673 nm/ms, about 96 times greater than the velocity of dynein motors in vivo. The forward translational movement of the body is coupled to both the rocking of the posterior cell body about its own axis and the axis of locomotion as well as the propagation of the bihelical waves and kinks. Our results demonstrate that millisecond-timescale microscopy is essential for studies of cell locomotion in microorganisms.

3254-Pos Board B301

Timing the Start of Division in E. coli: a Single-Cell Study

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Precise determination of morphology dynamics during growth and division of bacterial cells is restricted by optical resolution and micron size of the object. We have developed a method for high precision cell edge detection in a phase-contrast image allowing continuous follow up of the cell contour with about 30 nm accuracy. This approach is used to analyze the entire life cycle of single E. coli cells and provides a detailed morphological characterization of the cell division process. We show that initiation of the envelope constriction occurs much earlier than the appearance of a visible constriction, and is also manifested in a break in the length dynamics corresponding to the addition of new poles formation. We use simple rescaling of variables to provide a global view of the entire cell population. In particular, the data for the dynamics of the constriction width for all the cells in the population collapses to the vicinity of the function predicted by our theoretical model. Some of the parameters that describe cell division obey certain quantitative relations. In addition, we have developed an algorithm for analysis of the spatial distribution of the division initiator protein, tubulin-like FtsZ, in fluorescent images of single cells. With this algorithm, profile and positional dynamics of the FtsZ constriction ring were analyzed, revealing a time gap between the ring maturation and the start of constriction. This gap is presumably required for assembly of the other division proteins forming the divisome. This information provides new constraints on the possible molecular mechanisms involved in the formation of both the divisome and the cell septum.

3255-Pos Board B302

High-pressure Microscopy For Modulating The Torque Generation Of Bacterial Flagellar Motors

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The bacterial flagellar motor converts the specific ion flux across the cell membrane to the rotational motion. The torque generation is achieved by the intermolecular interaction between rotor and stator complexes. The motor can spin both directions; binding activated CheY molecules induces switching from counter-clockwise (CCW) to clockwise (CW). Here, we show a novel assay that changes the rotational speed and direction of the flagellar motor by specially designed high-pressure microscopy. *E. coli* cells lacking *cheY* that rotate exclusively in the CCW direction, were tethered by their flagellum to the observation window of high-pressure chamber. At less than 800 atm, all cells rotated in the CCW direction and their speeds were not affected seriously. At more than 1000 atm, some cells started to rotate in the CW direction, and the rotational speed in both directions decreased steeply with pressure. Application of pressure generally works to modify the intermolecular interaction between protein and water molecules, resulting in changing the structure and function of molecular machines. Thus, applied pressure seems to modify directly the intermolecular interaction between rotor and stator units. The pressure-induced effects could inhibit the torque generation of the flagellar motor, and change the rotational direction, as if the activated CheY molecules bind to the rotor.

3256-Pos Board B303

Enhancement of Bacterial Motility due to Speed-Dependent Absorption

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Marine bacteria often reach high swimming speeds, either to take advantage of evanescent nutrient patches or to beat Brownian forces. Since this implies that a sizable part of their energetic budget must be allocated to motion, it is reasonable to assume that some bacteria are able to increase their nutrient intake by increasing their speed v. We formulate a model that uses the concept of internal energy depot originally developed by Schweitzer, Ebeling, and Tilch to investigate this hypothesis. We postulate that the nutrient absorption rate is of the form $q(v) = q_0 + Av$, with q_0 and A being constants. If the fraction c of energy spent non-mechanically is low, we find that there is a single stable velocity v_1*, but if c is large, there is a critical value of A, A_c, below which only the $v = 0$ solution is stable. Above the bifurcation point A_c a second stable solution appears, whose value v_2* increases monotonically with A. The mechanical efficiency of the molecular motors is also shown to increase with A. The description of the motion is further clarified by the use of the Fokker-Planck formalism. Solutions obtained using realistic parameter values indicate that the speed increase due to the enhanced nutrient absorption may be substantial.

3257-Pos Board B304

Quantification of Leaf Vein Patterning

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Vein networks are essential in transporting nutrition effectively into all cells of an organism. In plant leaves these vein networks are formed by the opposite transport mechanism, the retraction of the plant hormone auxin. The so formed auxin flow pattern is consistent with the vascular network of the mature leaf. Key factor in the non-uniform transport are auxin carriers from the PIN protein family.

We investigate a microscopic model for the directed auxin transport by carrier proteins performing both computer simulations and analytic calculations. These enable us to identify the relevant biological processes which should be considered for leaf vein patterning. Quantitative results help us to suggest observables and experimental scenarios to measure the kinetic rates governing the active transport.

3258-Pos Board B305

Stochastic Effects On Biodiversity In Cyclic Coevolutionary Dynamics

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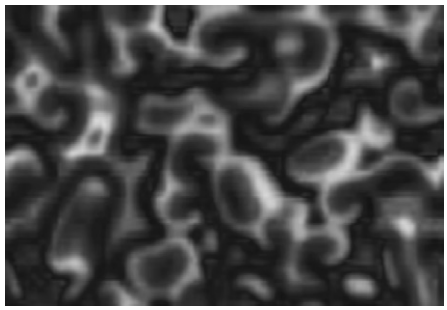
The formation of out-of-equilibrium patterns is a characteristic feature of spatially-extended, biodiverse, ecological systems. Intriguing examples are provided by cyclic competition of species, as metaphorically described by the 'rock-paper-scissors' game. Both experimentally and theoretically, such non-transitive interactions have been found to induce self-organization of static individuals into noisy, irregular clusters. However, a profound understanding and characterization of such patterns is still lacking. Here, we theoretically investigate the influence of individuals' mobility on the spatial structures emerging in rock-paper-scissors games. We have devised a quantitative approach to analyze the spatial patterns self-forming in the course of the stochastic time evolution. For a paradigmatic model originally introduced by May and Leonard, within an interacting particle approach, we demonstrate that the system's behavior - in



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)]

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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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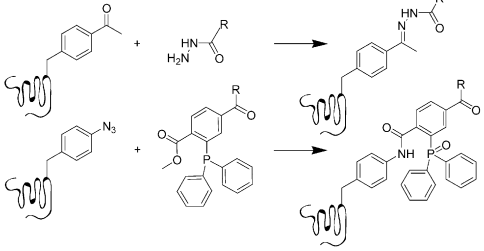
The insertion of unnatural amino acids into proteins using amber stop codon suppression has shown promise as a technique for probing protein structures. To investigate applications to studies of G protein-coupled receptors, we have developed methods that allow incorporation of each of three tyrosine 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

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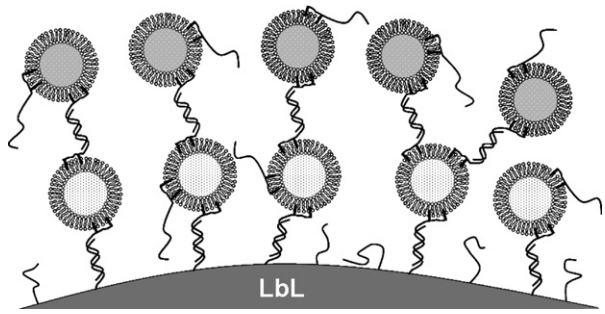
We report here on the formation of layers of large unilamellar vesicles (LUVs) superimposed on Layer-by-Layer- (LbL-) particles. DNA oligonucleotides were covalently attached to the outermost negatively charged polyelectrolyte layer of the particles and thus vesicles, with complementary lipophilic DNAs 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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