nal extensions (NTE) evolved independently in several branches of two major metazoan lineages, Protostomes and Deuterostomes. In some Protostomes (insects and flatworms) the regulatory light chains (RLC) have the NTE; in Deuterostomes (chordates and other phyla) only the essential light chains (ELC) of vertebrates have the NTE. Mechanical studies of insect (*Drosophila*) indirect flight muscle and vertebrate (mouse) myocardial strips, conducted at *in vivo* lattice spacing, indicate the NTE modifies cross-bridge kinetics. In flies, genetic deletion of amino acids 2–46 of the RLC NTE reduces the frequency of optimum cross-bridge oscillatory power. The reduced frequency can be modeled as a decrease in forward rate constant of the power stroke, resulting in a reduced number of force-generating myosin heads. In mice, genetic deletion of amino acids 5–14 of the ELC NTE increases the characteristic frequency of cross-bridge work production but reduces isometric tension. The frequency increase can be modeled as an increase in reverse rate constant of the power stroke, resulting in a reduction in both the number of myosin heads bound and generated force, thereby leading to a slightly hypertrophic heart as a compensatory response to reduced force. Both RLC and ELC phenotypes suggest the primary role of the NTE is to promote the formation of force-producing cross-bridges. The absence of structural abnormalities in the mutants is consistent with the emergence of the extensions being associated with fine tuning of the contraction kinetics.

909-Plat A Population Of Smooth Muscle Myosin With One Head Phosphorylated Has Half The Mechanical Activity Of Doubly Phosphorylated Myosin

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Smooth muscle myosin is fully active when the regulatory light chains on each of its two heads are phosphorylated (2P), and fully inhibited and in a folded conformation when both heads are unphosphorylated (0P). The inhibited state involves an asymmetric interaction between the two heads. Is phosphorylation of a single head (1P) sufficient to disrupt the inactive conformation and allow both the phosphorylated and unphosphorylated heads to be mechanically active? Using smooth muscle heavy-meromyosin (HMM) engineered to be exclusively 1P, we compared the mechanical activity of 1P to 2P HMM. We determined actin filament velocity in the motility assay under limiting myosin conditions, where velocity depends on the number of myosin molecules interacting with the actin filament. Then, using the optical trap, we compared force-velocity relationships for small ensembles of 1P and 2P HMM. In both assays, the 1P was slower ($p<0.001$) than the 2P HMM at equal concentrations. However, by doubling the concentration of 1P HMM, the 1P mechanical activity equaled that of the 2P HMM. To test whether both heads in the 1P HMM are partially active, we performed motility at saturating myosin concentrations and measured step-size and attachment time for single molecules in the optical trap. The 1P HMM had velocity (0.6 um/s), step-sizes (11 nm), and attachment times at 10 uM ATP (90 ms) indistinguishable from the 2P HMM, indicating that the active heads in the two preparations have similar mechanics and kinetics. We conclude that a population of 1P HMM has half the mechanical activity of 2P HMM, a result that could be explained either by an equilibrium between fully active and fully inhibited molecules, or by each molecule having only one mechanically active head.

910-Plat An In Vitro Model System For Determining Regulatory Mechanisms Of Smooth Muscle Mechanics

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The goal of this work is to develop an in vitro, regulated smooth muscle system for studying the mechanisms by which smooth muscle mechanics are modulated. Here we have developed a reconstituted, Ca2+-sensitive smooth muscle system incorporating physiological ratios (at least 20:1) of smooth muscle myosin (SMM) to myosin light chain kinase (MLCK) on a nitrocellulose-coated coverslip. We show that Ca2+ activates actin motion with a similar pCa observed in vivo. Using a novel approach we show that SMM is phosphorylated on the coverslip, but only in the presence of Ca2+. Phosphorylation was inhibited in the presence of wortmannin, an MLCK inhibitor. Trifluoroperazine (calmodulin [CaM] antagonist) treatment abolished Ca2+-dependent actin motion, which could be reconstituted on the coverslip by adding exogenous MLCK and CaM, but not either protein individually. Pre-treatment of the SMM with a peptide constituting the actin-binding domain of MLCK (which should dissociate MLCK if bound to the minute amount of actin present in the system) did not inhibit Ca2+-activated motion. This suggests that MLCK and CaM together form a tightly-bound, Ca2+-independent complex with SMM, not actin, and act as a functional complex that can be Ca2+-activated to phosphorylate SMM. We provide evidence that the MLCK:CaM:Ca2+ complex phosphorylates one myosin and then diffuses to other myosins to effect further phosphorylation, thus explaining the observed motion at the high ratio of SMM to MLCK.

Platform U: Biotechnology & Bioengineering

911-Plat Spatially Arranged Nonadhesive Surface Domains for Differentially Controlled Cell Adhesion

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Surface-patterned nanoscopic poly(ethylene glycol) [PEG] hydrogels can promote a wide range of cell responses. In contrast to conventional approaches of direct surface patterning and immobilization of specific adhesion-promoting molecules, the electron-beam patterning of PEG is a relatively simple and flexible technique for fabrication of nanoscopic hydrogels with defined dimensions and crosslink density. Here, we engineered surfaces that are selec-

tively adhesive to neural cell processes, such as neurites and astrocytic filopodia, based on the spatial density of individual nanohydrogels. At the highest density, there was no cell adhesion, consistent with the cell-repulsiveness generally attributed to PEGylated surfaces. As the spacing was increased to 2 microns, neurites were able to grow between the nonadhesive nanohydrogels. Astrocyte processes were unable to penetrate this same surface structure, however. At a spacing of 3 microns or more, processes from both neurons and astrocytes were able to adhere to the patterned surface to an extent approaching control levels. In addition, the density and average size of adherent astrocytes depended on the nanohydrogel spacing. We were furthermore able to engineer surfaces, specifically a series of parallel lines, which directed the growth of both neurons and astrocytes. Despite their minimal coverage of the total surface area, the patterned nanohydrogels controlled the elongation of the astrocytes and the precise position and direction of the growth of neurites. This method for creating differentially adhesive surfaces could be potentially used to engineer implantable devices for promoting neural regeneration while blocking formation of glial scar that inhibits growth of neurons.

912-Plat Scaffoldless Tissue-Engineering Using 3D Bioprinting: from Tissue Liquidity to Vascular Patterning

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Understanding the principles of biological self-assembly is essential for developing efficient strategies to build living tissues and organs. In this respect, the analogy between the behavior of immiscible liquids and embryonic tissues has allowed for a better understanding of phenomena such as establishment of tissue boundaries during early morphogenesis. The determinants of this behavior are the apparent tissue surface and interfacial tensions, which are global reflections of the intensities of cohesion and adhesion between the composing cells.

We exploited this analogy to engineer blood vessels that consist of three different cell types (Endothelial Cells, Smooth Muscle Cells and Fibroblasts) organized in distinct layers within the vessel wall. Surface tension measurements showed that endothelial cell spheroids were less cohesive than those composed of fibroblasts, which in turn, were less cohesive than those composed of smooth muscle cells. The outcome of engulfment and sorting experiments were consistent with the measured values of the tensions, the less cohesive cell type surrounding the most cohesive one. Based on these results we combined different pairs of cell types in single spheroids and used them as building blocks in the bioprinting process.

In this technology, multicellular spheroids (bio-ink particles) are placed into biocompatible environment (bio-paper) by the use of a three-dimensional delivery device (bio-printer). Three-dimensional scaffoldless tissue structures are formed through the post-printing fusion of the bio-ink particles. Using this technique, vascular tubes that possessed the 3 layers (intima, media, adventitia) found in native arteries were built. The geometrical parameters of those tubes like wall thickness, diameter, and branching patterns could easily be customized. Such constructs could fulfill the crucial need for small diameter vascular grafts and provide new strategies for vascularization of tissues for transplantation.

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913-Plat Electrophoretic Adsorption of Microtubules on Patterned Surfaces

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Selective adsorption of biomolecules on patterned surfaces has the potential for lab-on-a-chip applications and as a reconstituted system for investigating subcellular processes. We combined electron beam lithography, self-assembled monolayers (SAMs) and electrophoresis to develop a system in which controlled binding of microtubules was achieved on micron width gold tracks. The nonfouling polyethylene glycol SAM was formed to cover either the whole surface or only the non-patterned areas, depending on whether adsorption was to be made reversible or not. DC electric fields up to 5×10^4 V/m were tested, with the gold track as an anode. Electrophoresis spatially concentrates microtubule filaments onto these lithographically defined electrodes, while the SAM prevents nonspecific binding in the surrounding area. The speed and density of adsorption can be controlled by the applied voltage. This method has the advantage over other methods such as those using microfluidics or dip-pen nanolithography since arbitrary patterns can be generated and adsorption can be rapidly achieved over large areas.

914-Plat Single-molecule DNA Biosensors For Transcription-factor Detection

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Transcription factors are proteins that bind to specific regions on DNA and regulate gene expression. Detection of transcription factors can provide essential information about gene regulation, address fundamental biological problems such as development and cell-commitment, and lead to ultrasensitive diagnostics.

Here, we describe a single-molecule fluorescence assay that detects transcription factors with high sensitivity and specificity. The single-molecule assay is based on protein-detected coincidence of two DNA fragments (Heyduk & Heyduk, Nat Biotech 2002, *20*:171), each containing one half-site for transcription-factor binding; this coincidence is detectable using 2-colour alternating laser excitation (ALEX) spectroscopy (Kapanidis et al, PNAS 2004, *101*:8936). In the absence of a transcription factor that can bind to the fully assembled site, the two half-site DNA fragments (which carry short and complementary single-stranded DNA tails) diffuse independently in solution. In the presence of a transcription factor specific to the fully assembled DNA site, the two DNA fragments diffuse as a single molecular complex; such species can be distinguished from the free DNA half-sites and can be counted, reporting

on the presence and concentration of transcription factors. Using this assay, we demonstrated protein-dependent DNA coincidence to detect transcription factors in dilute (sub-nM) protein solutions; multiplexing capability by detecting two transcription factors simultaneously in the same solution; compatibility with complex biological samples such as nuclear extracts; and sensing of changes in gene expression in bacterial cells. Finally, we have shown that the assay can be implemented using a single immobilized DNA half-site and a complimentary half-site in solution; in this case the protein presence is monitored by fluorescence coincidence analysis using total internal reflection fluorescence microscopy. The assay can be extended for monitoring transcription factors in living cells and for detecting disease markers.

915-Plat Putting the Brakes on Kinesin-1 - Mechanisms for Selective Speed Control of Cargo Carrying Microtubules

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Biomolecular motors are promising tools for highly efficient molecular sorting and nano-assembly devices. Towards this goal, *in vitro* transport assays usually utilize surface-attached motor proteins to propel cytoskeletal filaments. Nano-sized cargo is then most commonly attached via a biotin-streptavidin linkage. While heavy cargo-loading has been reported to influence the speed of gliding filaments, it has not been investigated if this slow-down was caused by "frictional" cargo-surface interactions or whether the attached molecules act as roadblocks for stepping motors.

To answer this question, we performed systematic gliding motility experiments using kinesin-1 motors and biotinylated microtubules loaded with varying amounts of streptavidin. By monitoring their gliding speeds, we determined the critical streptavidin density above which microtubules slow down. In parallel, we studied the behavior of individual kinesin-1 motors on streptavidin-coated microtubules. We found that single kinesin-1 molecules frequently stop when they encounter streptavidin obstacles on the microtubule lattice. However, about half of the stopped kinesin-1 molecules were able to overcome these obstacles eventually.

We explain the stopping by kinesin-1's unique properties: Moving processively along individual microtubule protofilaments, kinesin is unable to walk around an obstacle or to detach immediately. However, if the obstacle and kinesin do not occupy the same binding site on the microtubule lattice, molecular flexibility will allow the molecules to pass each other. Our findings agree with kinesin-1 operating on highly crowded microtubules in vivo and emphasize the suitability of the kinesin-microtubule system for nanotechnological applications.

To demonstrate possible applications beyond optimized cargo-loading, we mixed microtubules labeled either with biotin or rhodamine. Adding streptavidin did then selectively slow down the biotinylated microtubules only, while anti-rhodamine antibodies slowed the rhodamine-labeled filaments. We propose to use this selective slow-down for molecular sorting and sensing applications.

916-Plat Directed Fluid Transport Driven by Magnetically Actuable Biomimetic Cilia Arrays

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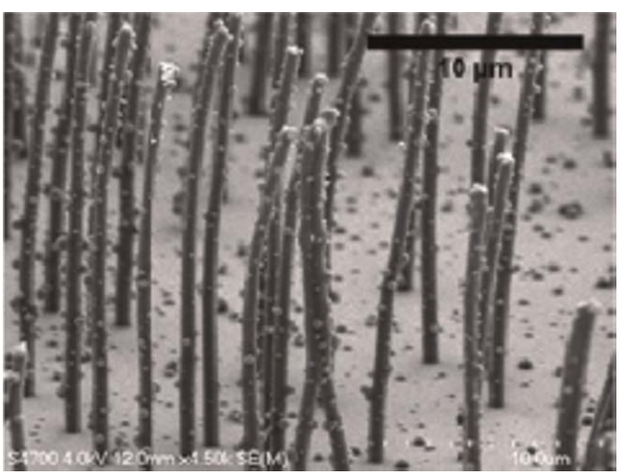
Cilia are ubiquitous in biology for generating fluid flows for motility and physiological function. However, much needs to be learned about cilia generated fluid flows in Newtonian and viscoelastic fluids. We present a technique for fabricating large arrays of soft polymeric microstructures similar in length (10 to 25 μm) and aspect ratio (10 to 125) to biological cilia. We have used this technique to fabricate arrays made of a novel maghemiteferrofluid / polydimethylsiloxane composite material. The magnetic component of the structures enables actuation via external magnetic fields, and we have demonstrated large-scale actuation of these arrays at frequencies as high as 30 Hz with magnetic fields produced by small NdFeB magnets. As part of the Virtual Lung Project at UNC Chapel Hill, we are currently using these biomimetic cilia to explore the fluid dynamics of mucociliary clearance in human lung epithelial cells. We have demonstrated directed transport of a viscoelastic fluid across a biomimetic cilia array, and have begun to characterize the conditions necessary for such transport to occur. These biomimetic cilia arrays may also find application as an active anti-biofouling surface, or have microfluidic, photonic, or sensing applications.



917-Plat A Kinesin-based Molecular Shuttle

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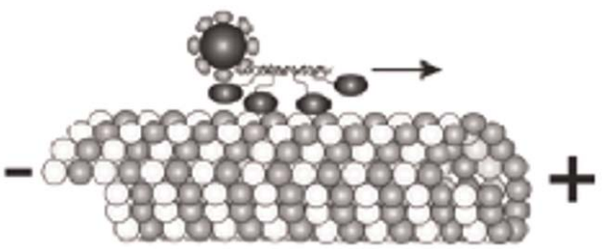
We have sought to harness the ubiquitous eukaryotic molecular motor protein kinesin to build an engineered molecular shuttle with a specific, programmable stoichiometry and geometry. By fusing zinc-finger DNA-binding motifs to kinesin heads, and using DNA

templates containing zinc-finger recognition sequences, teams with a defined number (1–6) of single-headed kinesin motor proteins have been assembled. This allows investigation of the effects of team composition, including the number of heads and the length and flexibility of the linkages between them, on the function of these composite motors.

Microtubule gliding assays show that these shuttles are functional: microtubules move across surface-bound teams, at a velocity similar to that measured with independent, single kinesin for monomeric to tetrameric populations at 24°C. Gliding in the temperature range 15–28°C has been investigated. The motion of single molecular shuttles labelled with quantum dots along static microtubules has also been observed by fluorescence microscopy. This allows measurement of velocity, dwell time and run length of quantum dots attached to single teams.

The use of a double-stranded DNA template has potential not only to specify nanoscale structure, but also to allow dynamic exchange of the protein arrangement, through the use of DNA strand exchange.



918-Plat Programmable Assembly of Vesicle Superstructures using Membrane-Anchored DNA as Biomolecular Combination Locks

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Much interest has been shown over the past decade in the programmable assembly of hard-sphere colloids mediated by the specific interaction between complementary ssDNA sequences. Lipid vesicles potentially offer greater levels of control in structural assembly due to some of their unique properties, including the deformability and fluidity of membranes, and the ease with which the properties of vesicles can be tuned by utilizing the multitude of lipids that are readily available. Due to the role of lipids in nature as biological packaging material, lipid vesicles have been considered for engineering applications as soft, technological containers. The ability to reversibly assemble vesicles into multicompartment containers has many applications in microfluidics and, potentially, drug delivery. We approach this innovative challenge by anchoring single-stranded DNA (ssDNA) to the outer monolayers of lipid vesicles: superstructures are observed to form between different populations of vesicles decorated with complimentary ssDNA strands. The fidelity of vesicle hetero-binding in the superstructures is found to be dependent on the strength of the DNA's hydrophobic anchor to the lipid bilayer. The aggregation is found to be reversible either by heating above the melting temperature of the DNA duplex or by the isothermal decrease of the ionic strength of the solution. Confinement of the DNA to the membrane surface greatly enhances the thermal stability of the double- stranded duplex compared to the stability in free solution. Furthermore, we can engineer DNA clustering by inducing lateral phase separation in multicomponent lipid vesicles. This causes the membrane-bound DNA to partition preferentially into one of the resultant phases, leading to the formation of localized 'sticky' patches on the membrane surface.

Symposium 9: RNA in Action

919-Symp Direct Measurement Of A pKa Near Neutrality For C75 In The Genomic HDV Ribozyme

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The hepatitis delta virus (HDV) ribozyme plays essential roles in the life cycle of the virus. Catalytic activity of the genomic HDV ribozyme is regulated by the binding of two protons and two magnesium ions. The protons bind to C75 and C41, and play catalytic and structural roles respectively. In addition, a magnesium ion binds in the vicinity of each cytosine. I will present solution ribozyme kinetics data that support intrinsic pKas for C75 of 5.9 and 7.2 in the presence and absence of bound metal ion, respectively, and intrinsic pKas for C41 of 7.1 and 5.5 in the presence and absence of bound Mg2+, respectively. In an effort to provide an independent physical measurement of the pKa, we have determined the pKa in crystals of the ribozyme using difference Raman spectroscopy. We grew crystals of the genomic HDV ribozyme bound to a non-cleavable substrate analog. Difference Raman spectra as a function of pH for the wild-type ribozyme provide a pKa of 6.2 in the presence of 20 mM magnesium ions. This value is similar to the pKa of 6.1 obtained in solution kinetics experiments under similar ionic conditions. Moreover, crystals of a C75U variant show no ionization behavior. The mutant data support assignment of this pKa to residue 75. Additional experiments show binding of metal ions in the crystals mirrors binding in solution, which further indicates that ribozyme in the crystal behaves like ribozyme in solution. Together these data show that ribozymes can use their active sites to optimize nucleobase pKa values for proton transfer, providing further support for the importance of general acid-base chemistry by C75 in the HDV ribozyme.

920-Symp Comparing to protein, RNA crystallization is a much more challenging task

Jing-Dong Ye[1], Valentina Tereshko[2], John K. Frederiksen[2], Akiko Koide[2], Frederic A. Fellouse[3], Sachdev S. Sidhu[3], Shohei Koide[2], Anthony A. Kossiakoff[2], Joseph Piccirilli[1,2]