invasion of hosts and disease initiation and progression. In order to monitor the pathogen invasion process, most often one mixes a solution containing pathogens into a culture of host cells, and then hopes the host cell being monitored is invaded by a pathogen. These chance encounters have been monitored using fluorescence microscopy, allowing for example fluorescent effector proteins to be monitored as they are injected into a host cell (Enninga, Mounier et al. 2005; Schlumberger, Muller et al. 2005). Such ''mix and hope'' strategies are simple, direct, and necessary for initial studies. However, they do not provide a path to obtain large sample sizes, control of timing of pathogen invasion, or a way to determine how many bacteria are required to defeat host defenses. We present recent results from using optoelectronic tweezers (OET) (Chiou, et al. 2005) as a tool for manipulating single pathogenic bacteria, opening a promising route for controlled initiation of HP interactions. Optoelectronic trapping uses laser-excited carriers inside a thin film of amorphous or crystalline Si to create a non-uniform electric field. These carriers, coupled with an electric field modulated in the MHz range produces strong field gradients. The object being trapped is polarized by the electric field, and gradients in the electric field create a potential well which traps the object. This trapping is obtained at extremely low optical intensities ($<1 W/cm^{\wedge 2}$) and does not require functionalization of the pathogen in order to facilitate delivery to the host, opening up novel possibilities for massively parallel studies of HP interactions.

3172-Plat

A Suitably Compliant Microenvironment Commits Mesenchymal Stem Cells to Differentiate into Muscle Like Cells Which Restore Muscular Defects in Dystrophic Models

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Human mesenchymal stem cells (hMSCs) express markers of different lineages when grown on matrices of an elasticity that mimics various tissues of mesenchymal origin. Of particular interest to our group is the possibility of using this approach to drive hMSCs into skeletal muscle lineage for repair of damaged skeletal muscle. In order to identify and determine the necessary *in vitro* culture conditions that will lead to optimal commitment of cells to the muscle lineage *in vivo*, we have been developing biophysical techniques to allow stem cell precommitment to a specific lineage prior to implantation in a diseased tissue by controlling matrix stiffness under *in vitro* culture conditions. This technology has led to a highly reproducible approach to optimizing stem cell fate for human transplantation. The physical nature of a cell's microenvironment - including elasticity of the surrounding tissue - appears to exert a significant influence on cell morphology, cytoskeleton and gene expression. Numerous gel systems - particularly polyacrylamide gels - have tunable elasticity that can be adjusted over several orders of magnitude from extremely soft to stiff, mimicking the elasticity of a wide range of tissues by controlling the extent of polymer cross-linking. Here, we use cross-linked polyacrylamide hydrogels that mimic the true *in-vivo* muscle-like elasticity (10-15 kPa) to induce hMSCs to differentiate into myoblasts expressing key early markers of muscle differentiation program (Pax7, myoD). They also fuse to form myotube like structures expressing late skeletal muscle markers like troponin I in vitro. We have recently used these committed cells on our *in vivo* animal models of muscular dystrophy and successfully demonstrated that they have a very high potential for integrating into skeletal muscle and rescuing the muscular defects.

3173-Plat

Single-Molecule DNA Biosensors for Quantitative Transcription Factor Detection

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We have developed two single-molecule fluorescence biosensors that can quantitatively detect single or multiple transcription factors (TFs) with high sensitivity and specificity. The first sensor is based on TF-based coincidence of two DNA fragments, each containing half of a particular TF binding sequence. In the absence of the particular transcription factor, the DNA fragments diffuse independently. The coincidence is detectable using 2-colour alternating laser excitation (ALEX) spectroscopy using either solution or surface-based approaches.

We have detected a single transcription factor (lactose repressor - lacR) at a concentration of 100pM without the need for amplification steps. Further we can detect TFs in a quantitative manner using a simple kinetic model, without the need for a calibration curve. Using an inducible plasmid for catabolite activator protein (CAP), we show quantitative detection of changes in gene expression in bacterial cell lysates over time.

We extend this first sensor to simultaneously detect two transcription factors (lacR and CAP) in the same solution. Two assays were designed to implement two basic Boolean logic operators (AND and OR). We demonstrate correct functioning of these operators using solution and surface-based approaches with nM TF concentrations. These operators can be cascaded to form arbitrarily complex intelligent sensing assays for true multiplexed detection of several TFs in one experiment.

The second sensor is based on the common phenomenon of TF-induced DNA bending. The design is uni-molecular with a donor-acceptor pair in close proximity and a binding site for the TF of interest. On binding of the TF (CAP), the DNA is bent, increasing the donor-acceptor distance. This can be detected via a change in FRET (Förster Resonance Energy Transfer) in a quantitative manner. Current investigations are focused on using this sensor for TF detection in cell lysates and in-vivo.

3174-Plat

A Microfluidic Device to Maintain Islet-Associated Endothelial Cells During Long-Term Tissue Culture

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Endothelial cells (EC) are integral to the characteristically dense vasculature of pancreatic islets. This vasculature enables accurate blood-glucose sensing and rapid secretion of insulin into the blood stream. It also provides pro-survival hemodynamic signals to EC. How EC and beta cells interact to affect glucose stimulated insulin response is an actively debated topic. However, long-term studies in the *ex vivo* tissue are limited by the loss of EC over a period of days in traditional culture. We postulate that the EC die in part from an absence of the shear and media exchange provided by hemodynamic fluid flow. To test the role of hemodynamic forces on EC, we created a microfluidic device capable of supplying a range of fluid flow to *ex vivo* islets. Our protocol controls temperature, pH and bubble formation using two hot plates and a syringe pump for long-term desk top experimentation. Using this microfluidic device with immunofluorescence microscopy, we examined the morphological response of islet-EC to a variety of flow rates for 24 and 48 hours. Our results show more than twice the average percent area and connectivity of EC in islets treated in the device as compared to no-flow controls stored in traditional cell culture. Using this device with varying media viscosity, we determined that the differences in morphology are due to media exchange and not shear-activated survival. As well, we are currently evaluating the effect of fluid flow on beta cell survival by ensuring normal glucose stimulated calcium and insulin response. Overall, our data indicates that flow in a microfluidic device provides a reliable *co-culture* environment enabling the long-term study of cell biology in the pancreatic islet.

3175-Plat

Engineering Lipid Bilayer Platforms for High Throughput Cell-Free Electrophysiology

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Ion channels are crucial physiologically, involved in nearly all biological processes. Their many roles make them important drug targets as well as targets to avoid, as for drug safety screening (e.g. the hERG channel). Patch clamp provides the highest quality measurements of ion channel function and is used by the pharmaceutical industry to test drug interactions with ion channels, but has poor cost and throughput performance. As an alternative ion channel measurement platform, artificial lipid bilayers are well established to provide a highly controllable environment capable of measurement at the single molecule level, although they also suffer from their own technological shortcomings. Previously, we developed an artificial lipid bilayer platform which is capable of shipping and indefinite storage. Here we present the continued technological development of this system which has resulted in faster bilayer formation times and the ability to package, store, and ship ion channels with the bilayer chip. We have also begun to integrate array chip designs with automated and high throughput solution manipulation and ion channel measurement hardware, advancing this platform closer to operator-free involvement and low cost high throughput cell-free electrophysiology.

3176-Plat

Towards Dark Quencher Based Real Time DNA Sequencing

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Third-generation DNA sequencing technologies are expected to transform biomedical research and health care. Although powerful single-molecule DNA

sequencing methods are available, they suffer from significant limitations, including the need for sophisticated instrumentation, microfluidics, or nanofabrication. Here, we introduce a simpler approach based on fluorescently labelled DNA polymerases and dark quencher labelled nucleotides (dNPP-Q, pentaphosphates). During the time between the binding and incorporation of a nucleotide, the fluorescence intensity of the polymerase-attached fluorophores is differentially reduced due to Foerster resonance energy transfer to a nucleotide-specific dark-quencher attached to the terminal phosphate group. Use of dark-quenchers enables real-time sequencing with long read lengths at micromolar nucleotide concentrations, and is compatible with standard total-internal-reflection fluorescence microscopy.

We first characterized dark quenchers for single-molecule detection by studying a set of double-stranded DNA constructs labelled with two fluorophores (''green'' and ''red'') and one dark-quencher. We then designed a biochemical system that allowed us to observe directly individual events of dNPP-Q binding to a binary complex of a DNA polymerase (labelled with a green fluorophore) bound to a primer-template substrate labelled with a red fluorophore. Binding events of dNPP-Q to the complex results in real-time observations of clear, transient reductions of green and red fluorescence by 80% and 30%, respectively; the quenching efficiency matches to the expectations based on the photophysical properties of the interacting chromophores and their separation. We are currently extending the concept to the remaining bases. Our approach should facilitate the study of DNA- and RNA-polymerase mechanisms as well as the development of faster and cheaper methods for single-molecule DNA sequencing.

3177-Plat

Photo-Control of Protein Activity in a Single Cell of a Live Organisim

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We take a multidisciplinary approach to realize a noninvasive optical control of the protein activity in live zebrafish embryo with single cell resolution. Our method relies on three components 1) the dependence of biological activity of the protein fused to estrogen receptor on the binding state of the estrogen to its chaperone, 2) the chemical inertness and the permeability of the cell membrane to the non-endogeneous caged inducer and 3) the selective optical uncaging of the inducer in the zebrafish embryo, globally or in a single cell using one or two photon microscopy. We believe that our method is very general and could be used to control the activity of large array of proteins. As a pilot study we used our method to activate 1) the nuclear translocation of two different fluorescent proteins in embryo and cell cultures, and 2) the Cre recombinase activity in an appropriate transgenic animal to genetically label a single cell of the embryo and cell lines. The ability of our method to change the genetic map of the selective cells in the embryos could be used more generally to investigate important physiological processes (for example in embryogenesis, organ regeneration and carcinogenesis) with high spatio-temporal resolution (single cell and faster than minute scales).

3178-Plat

Genetically Encoded Singlet Oxygen Generator (SOG) Requiring No Exogenous Cofactors

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Controlled local photogeneration of singlet oxygen (1O2, the metastable excited state of O2) is useful for generating electron-microscopic contrast, rapidly inactivating proteins of interest, reporting protein proximities over tens of nanometers, and ablating cells by photodynamic damage. The best previous genetically targetable SOG was the biarsenical dye ReAsH bound to tetracysteine motifs, but this system has modest quantum efficiency (0.024), requires antidotes to prevent toxicity, is limited by background staining, and is difficult to extend to intact multicellular organisms. We now report that Arabidopsis phototropin, a blue light photoreceptor containing flavin mononucleotide (FMN) as its chromophore, can be engineered into a small (106-residue) SOG (''miniSOG''), which absorbs maximally at 448 and 473 nm with extinction coefficients of 16,700 and 13,600 M-1cm-1 respectively. Quantum yields for fluorescence and 1O2 generation are 0.30 and 0.47. MiniSOG binds endogenous FMN very tightly (dissociation constant ~ 10-10 M), so bacteria and mammalian cells upregulate their total FMN to keep miniSOG saturated, without any obvious toxicity in the absence of illumination. Although the green fluorescence of miniSOG is weak and bleachable, it shows that fusions of miniSOG to a variety of proteins in mammalian cells appear to localize correctly, even inside organelles when appropriate. After fixation, illumination of miniSOG to generate 1O2 efficiently polymerizes diaminobenzidine into an osmiophilic deposit, enabling correlative electron microscopy. In an initial biological application, electron microscopy shows that a cell-adhesion molecule, SynCAM1, fused to miniSOG, predominantly localizes to the presynaptic side of cortical neuron synapses. This compact SOG relying only on ubiquitous endogenous FMN will greatly expand the utility of imaging and ablation techniques based on 1O2.

Platform AV: Microtubular Motors

3179-Plat

The Neck Linker Docking is Not Required for Kinesin-1 to Take a Step Forward

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Kinesin-1 moves processively along microtubules by alternatively moving two motor domains, but the mechanism of the preferential forward stepping is still controversial. The ''neck linker-docking model'' proposes that the neck linker of the microtubule-bound head propel the tethered head forward. We proposed an alternative ''biased-capturing model'' that the tethered head freely diffuses and is captured preferentially at the forward-binding site. The latter model predicts that the neck linker of the tethered head, not of the microtubule-bound head, is essential for stepping, but it is difficult to distinguish these effects using ''symmetric kinesin dimer''. To distinguish these models, we engineered ''asymmetric two-headed monomer'' kinesin. We joined two monomer heads tandemly on a single polypeptide, in which the neck linker of first head (Nhead) is connected to second head (Chead) so that it can propel Chead forward, whereas the neck linker of Chead is free and is not connected to Nhead. The neck linker-docking model would predict this mutant could not take the second step. Surprisingly, the two-headed monomer showed robust and unidirectional movement along microtubules in single molecule fluorescent assays. The distance travelled was even longer than wild-type dimer but the velocity was reduced by a factor of 4. Single molecule FRET observation showed that the mutant spent most of the time in a two-head-bound state where the Nhead is leading. These results indicate that the rate-limiting step of the two-headed monomer's processive movement is the forward stepping of Chead driven by the neck linker-docking of Nhead and that the neck linker-docking independent forward stepping of Nhead is rapid and more efficient. These results rule out the idea that neck linker docking is essential to take a forward step and favour the biased-capturing mechanism.

3180-Plat

Possible Intermediate States in the Microtubule Minus-End Directed Movement of the Ncd Stalk

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The walking step of the microtubule minus-end directed kinesin motor Ncd involves about 75-degree rotation of its coiled-coil stalk. Two crystal structures, 1CZ7 and 1N6M (protein data bank IDs) are related respectively to conformations before (plus-end pointing stalk) and after (minus-end pointing stalk) this movement. Using targeted molecular dynamics we traced the stalk motion between these two structures. We observed that the motor head first rearranges from the 1CZ7 to 1N6M conformation, followed by the travel of the stalk. The 75-degree rotation of the stalk is accompanied by a nearly 20-degree torsion in its coiled coil. Further, the stalk travel can be divided into sub-steps between intermediates at about 17, 38 and 56 degrees from the 1CZ7 conformation that are characterized by the breaking and forming of new salt bridges by Arg335 or Lys336 in the stalk with the charged groups along the alpha-1 domain of the motor head, some of which are not present in the crystal structures. These results suggest that when Ncd is making a step towards the microtubule minus-end, the motor head first changes conformation in a nucleotide-dependent manner that promotes the detachment of the plus-end pointing stalk. This is followed by the stalk rotation that may be broken down into sub-steps between the intermediate states. In case of a diffusive motion, this may be more effective than diffusion over the entire 75-degree range.