engineering typically focus on improved affinity or stability. Here, we examine methods to engineer coupled equilibria that are linked to the protein binding event, which result in protein variants that can be regulated reversibly, based on environmental conditions. Using single domain (VHH) antibodies as model affinity reagents, along with combinatorial libraries, which provide a route to screen new and wild-type residues across a significant amount of interface surface area, we explore the creation of either pH- or metal ion-dependent protein recognition. The resulting protein variants have been analyzed to evaluate the structural and thermodynamic consequences of the remodeled interfaces on protein regulation and stability. The results suggest the ability to introduce new function, such as a reversible linked binding event, is likely to scale with the complexity/size of the protein-protein (ligand) interface. Furthermore, the combinatorial approach to introduce new function should be generally transferable to other protein affinity reagent scaffolds.

3165-Pos Board B595

A Continuous-Flow C. elegans Sorting System with Integrated Optical Fiber Detection and Laminar Flow Switching

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Sorting of C.elegans genotypes is a routine task in most C.elegans research labs. Conventionally, the worms are sorted manually which is both labor intensive and slow. In recent years microfluidics has become a useful and important tool for biologists to study C.elegans, including sorting. Although they have shown successful sorting results, the immobilization of worms which was adopted in most of these works causes aversive stimulation to the worms and their behavior will potentially be altered when carrying out post-sorting characterizations.

In this work, both worm detection and switching are achieved without any intervention of the continuous worm flow. The genotypes of the worms are detected by integrated optical fibers based on their fluorescence, without the need for immobilization. Switching is based on the steering of laminar flow boundaries. A novel design that integrates two control inlets dynamically switches the fluidic flow to desired outlets by changing the relative pressure in the control inlets, which cause the two laminar flow boundaries to steer.

Compared to previously reported microfluidic C. elegans sorting devices, sorting in this system is conducted in a continuous flow environment without any immobilization technique or need for multilayer mechanical valves to open and close the outlets. The continuous flow sorter not only increases the throughput but also avoids any kind of invasive or possibly damaging mechanical or chemical stimulus. We have characterized both the detection and the switching accuracy of the sorting device at different flow rates, and efficiencies approaching 100% can be achieved with a high throughput of about 1 worm/s. To confirm that there was no significant damage to C. elegans following sorting, we recovered the sorted worms, finding no differences in behavior and propagation compared to control.

3166-Pos Board B596

Discovering Emergent Behavior of Host-Microbiome Interactions with Biomimetic Robotics

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Although underlying dynamics governing host-microbiome interactions are poorly understood, recent studies have shown that commensal bacteria play an important role in regulating the health and behavior of their host. In order to better elucidate this interaction, we designed a biomimetic robotic host platform comprised of an onboard synthetically engineered microbiome. By computationally simulating engineered gene networks in these commensal communities, we discovered complex emergent behaviors in the host, such as stalk-and-strike hunting patterns, dependent exclusively on biochemical network dynamics. This simulation models behavior at multiple scales from the molecular kinetics of genetic transcription to the physical actuation of robotic components. Taken as a whole, this study provides both a computational tool for understanding inter-kingdom communication while presenting a design for a biomimetic system capable of translating genetic based cellular behavior into macro-scale robotic locomotion.

3167-Pos Board B597

Regulation of Cell Function via Extracellular Biophysical Environment: A Theoretical- Experimental Approach

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Regulatory promise of electric field (EF) as a non-pharmacological, non-invasive tool to control cellular functions is of great therapeutic interest. However, biophysical mechanisms for the cell-EF interactions are not understood. We developed a theoretical-experimental approach to investigate EF effects on cells in electrode-free physiologically-relevant configuration, i.e. with cells attached to a substrate. Cell is modeled as a membrane-enclosed hemisphere with realistic parameters. Our numerical results demonstrated that EF frequency is the major parameter that controls the mechanism of EF interactions with cells in realistic environment. Non-oscillating or low-frequency EF leads to charge accumulation on the cell surface membrane and results in field screening from the cytoplasm, suggesting that in this regime, cell responses are regulated by EF interactions with the surface membrane receptors. In contrast, high-frequency EF penetrates the cell membrane and reaches cell cytoplasm, where it may directly activate intracellular responses. Theoretical simulation predicts the non-uniform distribution of the induced field within the cell membrane, which depends on the applied EF frequency. Importantly, substrate properties significantly affect both magnitude and distribution of the induced field on the cell membrane, underscoring the need for a comprehensive, physiologically-relevant modeling approach for EF-cell interactions. These theoretical predictions were confirmed in our experimental studies of the effects of applied EF on responses of vascular cells. Results show that non-oscillating EF increases vascular endothelial growth factor (VEGF) expression while field polarity controls cell adhesion rate. High-frequency, but not low-frequency, EF provides differential regulation of cytoplasmic focal adhesion kinase and VEGF expression depending on the substrate, with increased expression in cells cultured on RGD-rich synthetic hydrogels, and decreased expression for basement membrane (matrigel) culture. These results advance our understanding of complex mechanisms underlying cell-EF interactions and may contribute to future EF-based therapies.

3168-Pos Board B598

Mechanobiology of mRNA Localization in Breast Cancer Cells

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Introduction: Metastasis occurs when cancer cells form secondary tumors in distant areas of the body. Localization of RNAs in lamellipodial regions has been proposed to play an important role during metastatic progression. In one pathway, the tumor suppressor protein, adenomatous polyposis coli (APC) targets RNAs to cell protrusions. APC -associated mRNAs have been implicated in cellular migration and metastatic progression. Therefore, localization or not of these mRNAs has functional significance in cellular migration and metastasis. Additionally, It has been shown that cancer cells modulate their gene expression in response to the mechanical properties of the substrate. Furthermore, mRNA localization at adhesion sites is influenced by mechanical tension, which is adjusted by cells as a function of the mechanical properties of the cell environment. Therefore, mechanical properties of tissues may play a role during metastasis by modulating localization of mRNAs. As a result, this study investigates APC- associated mRNA localization as a function of substrate stiffness.

Methods: We used the MCF10A cell series, a breast cancer progression model composed of cell lines representing pre-malignant to invasive transformation to investigate mRNA localization. By using in situ hybridization to fluorescently label mRNAs, and micropatterned polyacrylamide gels of varying stiffness, we observed APC associated mRNA localization. Glu-tubulin and vinculin were immunostained to study their relationship to mRNA localization.

Results: On stiffer substrates (280kPa), we observed increased mRNA localization compared to softer substrates (0.87kPa). Staining of cytoskeletal elements such as Glu-tubulin and vinculin showed a correlation between the abundance and location of the proteins and APC-associated mRNAs. These