2094-Pos
Microscale Colocalization of CD3 and CD28 is Required for Activation of Human CD4+ T Cells
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It is increasingly recognized that intracellular cell signaling is dependent on the spatial organization of signaling molecules. We previously introduced a platform for investigating spatially-dependent signaling, in the context of the immune synapse, a small (~70 square micrometer) region of contact between T cells and Antigen Presenting Cells. Multiple rounds of microcontact printing are combined to produce glass surfaces with independently defined, microscale patterns of antibodies to CD3 (part of the TCR complex) and CD28 (a major costimulatory signal), surrounded by ICAM-1; antibodies locally engage and activate their respective ligands, organizing signaling complexes in cells on these substrates. We demonstrated that Il-2 secretion by naïve mouse CD4+ T cells is sensitive to the position of CD28 signaling within the region of cell-substrate contact, analogous to the immune synapse. Mouse T cell activation is less sensitive to the organization of CD3 both within the cell-substrate interface and with respect to the location of CD28. In sharp contrast, we demonstrate here that Il-2 secretion by human CD4+ T cells requires colocalization of CD3 and CD28 signaling. All patterns we examined in which antibodies to CD3 and CD28 are separated by micrometer-scale distances were ineffective in inducing Il-2 secretion. Immunohistochemcial staining using phospho-specific antibodies after 15 and 60 minutes following cell-substrate binding revealed that colocalized patterns are more effective than segregated counterparts in maintaining Lck phosphorylation at Y394, a site associated with full activation of this kinase. No differences in Zap70, PI3K, or PKC-theta were observed as a function of pattern geometry. Together, these results identify a dramatic difference between mouse and human T cell physiology, and suggest that Lck may be responsible for spatial integration of CD3 and CD28 signaling.

2095-Pos
Monte Carlo Study of B-Cell Receptor Clustering by Antigen Cross-Linking
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B cell signaling is triggered by the recognition of antigens by the surface proteins of the cell known as B-cell receptors (BCRs). It is known from experiments that, in the presence of soluble antigens BCRs assemble into small micro clusters and then structure into a macro cluster. However the underlying mechanisms of the antigen interaction with the BCRs and their cluster formation remain unclear. In our recent effort we have investigated, using Monte Carlo simulations, the mechanism of BCR clustering which would arise due to the intrinsic attractions among them. Such mutual attraction between two adjacent BCR molecules could arise, among other possibilities, due to cross-linking by bivalent soluble antigens. Recently, we have developed and studied a Monte Carlo model of B cell receptor clustering caused by binding and cross-linking of soluble antigens. The results of our study demonstrate the formation of small micro-clusters of BCR molecules (typically of size 2-10 molecules). But antigen cross-linking only is not adequate enough for the formation of large macro-clusters. A simple model of biased diffusion where BCR molecules experience a biased directed motion towards the largest cluster is then applied, which results in a single macro cluster of receptor molecules. The types of receptor clusters formed are analyzed using various network-based metrics such as the average distance between any pairs of receptors and number of adjacent pairs. The effect of BCR and antigen concentrations on the receptor clustering, the stability of the formed clusters over the time, and size of BCR-antigen cross-linked chains are all analyzed using suitable network-based metrics.

2096-Pos
Amplification & Analysis of the Synechococcus Os-B' Crispr Region from Single Cells
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The Octopus Hot Spring at Yellowstone National Park, which can reach near-boiling temperatures at its surface, is home to a microbial mat that has been found to be a good model for microbial diversity [90]. Among the many species of prokaryotes found in the hot spring mat are thermophilic strains of *Synechococcus*, which can be found in the photic zone at locations that correspond to a wide range of temperatures [2]. Fluorescence measurements of vertical mat slices have revealed a large degree of heterogeneity in the *Synechococcus* populations [3].
Conventional methods of genetic analysis require axenic cultures of bacteria, but less than 1% of bacteria species can be cultured in such a fashion [4]. To examine the genetics related to the aforementioned heterogeneity at the single-cell level, we have utilized and adapted a microfluidic chip for multiple displacement amplification (MDA) of DNA, which can amplify the DNA of a single cell for off-chip PCR and subsequent analysis [5,6]. Specifically, we are interested in examining the variation of the clustered regularly interspaced short palindromic repeats (CRISPR) region of the *Synechococcus* species found in the mat.
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2097-Pos
Cavitation Bubble Based Measurement of Red Blood Cell Elasticity
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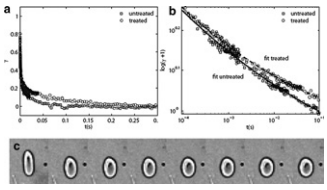
We present a novel technique to measure the red blood cell's (RBC) elasticity by exposing RBCs to an impulsive and transient flow. The flow is created from a rapidly expanding laser-induced cavitation bubble. The expanding bubble leads to a stretching deformation of cells close by. The fast flow lasts for approximately 20 microseconds and quickly ceases afterwards. Thereafter the RBCs relax back to their original shape, however on a much slower timescale. This relaxation is studied with high-speed photography and analyzed with digital image processing. In particular we determine the relaxation of the RBC's major and minor axis and find excellent agreement with a power law over several decades.
We compare findings on the elasticity of RBCs treated with neuraminidase and confirm the results from the common but much more laborious aspiration method. The figure above depicts the measured strain in linear and logarithmic scaling of treated and untreated RBCs, and a typical relaxation process of a single cell as a function of time.



The advantages of this novel technique are its simple implementation and the study of many cells simultaneously.

2098-Pos
Mechanical Properties of Desmin in Skinned Fibers from Normal and *desmin-Null* Mice
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Biomechanical properties of desmin, an intermediate filament protein, and its links through costameres to the contractile apparatus and the subsarcolemal cytoskeleton in single mammalian myofibers of *Extensor digitorum longus* single myofibers isolated from wild (**WT**) and desmin-null (**des-/-**) mice were measured. Suction pressures (**P**) applied through a pipette to the sarcolemma generated a bleb, the height of which increased with increasing **P**. At large **P**s, the connections between the sarcolemma and myofibrils broke; eventually the sarcolemma itself burst. We determined the values of **P** at which these changes occurred, and used these to determine the tensions and stiffness of the system and its individual elements. Tensions of the whole system and the maximal tension sustained by the costameres were (1.6-fold) lower in **des-/-** muscles than in **WT**. Separation and bursting **P**s, as well as the stiffness of the whole system and the isolated sarcolemma were ~1.4-fold lower in **des-/-** than in **WT**. The viscoelastic parameters of the entire system and costameres were also reduced in desmin-null myofibers. Our results indicate that the absence of desmin reduces muscle stiffness, increases sarcolemmal elasticity, and compromises the mechanical stability of costameres and their connections