999-Pos
Inhomogeneous Morphology and Elasticity of Mouse Oocyte Zona Pellucida Pre- And Post-Fertilization
Svetlana Kotova[1], Maria Jimenez-Movilla[2], **Emilios K. Dimitriadis[1]**, Jurrien Dean[2].
[1]LBPS, NIBIB, NIH, Bethesda, MD, USA, [2]LCDB, NIDDK, NIH, Bethesda, MD, USA.
The mature mammalian oocyte is encapsulated within a thick membrane, the zona pellucida (ZP), which is composed of a small number of glycoproteins. The proteins are secreted by the oocyte during maturation. Sperm has to penetrate the ZP first before it reaches the oocyte. The ZP controls for species-specific fertilization and acts as the barrier to polyspermy, i.e. it prevents a multiplicity of sperms from reaching the oocyte. All ZP proteins contain a characteristic ZP domain but, otherwise, their 3-D structure is unknown as is the architecture of the zona membrane. Upon fertilization, one of the proteins (ZP2) is cleaved near its N-terminal. The cleavage is believed to alter the ZP structure which, henceforth, acts as a barrier to further sperm penetration.
We used the atomic force microscope (AFM) to examine both the ZP structure and mechanical properties of the wildtype mouse ZP under physiological conditions. For that purpose, patches of isolated mouse ZP were immobilized on polylysine coated mica. Imaging revealed two predominant membrane surface morphologies consistent with previously reported electron microscopy images of the outer and inner membrane surfaces, respectively, from different species. One is a rough, ruffled surface and the other is a smoother surface with the appearance of a tighter construction. In addition to the surfaces, the structure across the wall thickness was visualized at high resolution revealing a layered, well organized architecture. Elastic modulus estimates from force-indentation data also showed systematic variability mirroring the morphological inhomogeneity. For example, significant differences in elasticity were measured between the rough regions, hypothesized to be outer surfaces, and the smoother regions. Interestingly, these properties also appear to undergo appreciable changes upon fertilization pointing to the structural change effected by the ZP2 cleavage.

Nano & Microfluidics, Biosensors

1000-Pos
Understanding the Stretching of DNA Molecules Confined in Nanofluidic Channels
Kyubong Jo[1], Yoori Kim[1], Gun-Young Jung[2].
[1]Sogang University, Seoul, Republic of Korea, [2]GIST, Gwangju, Republic of Korea.
Nanofluidic controls of single DNA molecules have provided a new approach of dynamically stretching large DNA molecules. Stretched DNA molecules enables single-molecule schemes aimed at the acquisition of sequence information. Also, nanoconfined DNA molecules provide opportunities to understand mechanistic details that used to be only plausible in theoretical considerations. Here we present the longest DNA molecules stretched in nanochannel ever reported: 20.0 μm out of 21.8 μm of YOYO-1 intercalated lambda DNA which is 92% of polymer's full contour length in PDMS nanoslits. In addition, we measure these elongations in various dimensions and reduced ionic strength to facilitate DNA elongation. Finally we compare our observations with theoretical predictions recently developed.

1001-Pos
Controlled Synthesis of DNA Nanocomplexes in a Microfluidic Device
Yi-Ping Ho[1], Yajun Duan[2], Feng Zhao[1], Kam W. Leong[1].
[1]Duke University, Durham, NC, USA, [2]Nankai University, Tianjin, China.
Nucleic acid-based therapeutics have emerged as a promising class of drugs but require a safe and efficient delivery system to realize their full therapeutic potential. While nonviral vectors may be safer than viral vectors in intracellular delivery, the need to improve their delivery efficiencies has provided the impetus to control the structural and chemical properties of DNA nanocomplexes. A commonly adopted approach to synthesize DNA nanocomplexes in nonviral delivery is to complex DNA with a gene carrier via electrostatic self-assembly, facilitating cellular uptake of DNA while protecting it against degradation. This poorly controlled bulk mixing technique, however, generates highly heterogeneous nanocomplexes in size and composition, hindering the establishment of structure-function relationship. The poor quality of these nanocomplexes is a significant impediment to the advance of nonviral gene delivery. The concept of miniaturization has been proposed for biological and chemical analysis for the past two decades. Of particular note has been the development of microfluidic technologies or "lab-on-a-chip" applications. Microreactors offer new opportunities due to the enhanced heat/mass transfer, low power/sample consumption, low production cost, high throughput synthesis and screening, and parallel sample processing. Herein, we present a controlled synthesis of DNA nanocomplexes in a microfluidic droplet generator. An individual droplet is ideally suited to compartmentalize and confine the DNA and gene carrier solutions. Further, localization of reagents within discrete droplets is an effective way to minimize the dispersion and loss of reacting volumes, which allows precise control of the reaction. This study focuses on the synthesis of DNA nanocomplexes, but the developed technology would also be applicable for other nucleic acid-based payloads, such as aptamer and siRNA.

1002-Pos
Design of Biosensors Based on the Covalent Assembly of G-Protein Coupled Receptors and Potassium Channels
Lydia N. Caro, Christophe J. Moreau, Jean Revilloud, Julien P. Dupuis, Michel Vivaudou.
Institut de Biologie Structurale, Grenoble, France.
It is possible to achieve functional coupling between a receptor and an ion channel by covalent linkage so that ligand binding to the receptor modifies channel gating. This was demonstrated with the inward rectifier potassium channel Kir6.2 (the pore subunit of the K_{ATP} channel) and the muscarinic M2 and dopaminergic D2 G-protein coupled receptors (GPCRs) [Moreau et al., 2008, Nature Nanotech]. To extend this concept of Ion-Channel Coupled Receptor (ICCR), we designed new contructs by engineering fusion between Kir6.2 and 3 GPCRs: the $ß_2$ adrenergic, cannabinoid 1 (CB1) and dopaminergic D3 receptors. The receptor C-ter and channel N-ter extremities were pared to promote efficient coupling as in M2 and D2 ICCRs and joined covalently. The fusions were heterologously expressed in *Xenopus* oocytes and characterized by the two-electrode voltage clamp technique. Construct names 'G-K_{xx-yy}' indicate the GPCR name (G), the residues clipped off from the GPCR C-ter (xx) and from the Kir6.2 N-ter (yy). A D3-based ICCR, D3-K_{0-25}, behaved like the D2-based ICCR, showing channel inhibition upon dopamine application. Two $ß_2$-based ICCRs were successfully constructed, $ß_2$-K_{62-25} and $ß_2$-K_{73-25}. Only when co-expressed with TMD0 (the Kir6.2-anchoring domain of SUR, the regulatory subunit of the K_{ATP} channel) to augment surface expression, these two ICCRs were reversibly activated by the agonist isoproterenol and inhibited by the antagonist alprenolol. Similarly, a CB1-based ICCR, CB1-K_{0-25}, was activated by the agonist W102, an effect that was enhanced by the presence of TMD0.
Thus, the ICCR concept is readily applicable to class A GPCRs. Besides their obvious interest in drug screening, the new ICCRs should be valuable tools to investigate the intermolecular events involved in the modulation of Kir6.2 gating and the nature of the GPCR conformational changes evoked by their ligands.

1003-Pos
Broadband Dielectric Spectroscopy of Bovine Serum Albumin and Insulin Solutions in Nanoliter Volumes
Nathan D. Orloff[1,2], Jaclyn R. Dennis[3], Ichiro Takeuchi[4], James C. Booth[1].
[1]National Institute of Standards and Technology, Boulder, CO, USA, [2]Department of Physics, University of Maryland, College Park, MD, USA, [3]Department of Molecular, Cellular, and Developmental Biology, University of Colorado, Boulder, CO, USA, [4]Department of Materials Science and Engineering, University of Maryland, College Park, MD, USA.
We perform quantitative frequency-dependent dielectric measurements of bovine serum albumin and insulin at varying concentrations using nanoliter measurement volumes. Bovine serum albumin solutions are in buffered water at 1 mg/mL, 10 mg/mL, 20 mg/mL, and 40 mg/mL concentrations. Insulin solutions are in HEPES (4 - (2 - hydroxyethyl) - 1 - piperazineethanesulfonic acid) at concentrations of 1 mg/mL, 5 mg/mL, and 10 mg/mL. A coplanar waveguide is used to extract the frequency response from 1 GHz to 40 GHz. An interdigitated electrode is used to measure the frequency dependence of the permittivity from 100 kHz to 1 GHz. The measurements are carried out in a 200 micron wide microfluidic channel defined by 50 micron thick SU-8 side-walls and capped with polydimethylsiloxane roof. The conductivity per mL/mg for insulin and bovine serum albumin is 0.24 uS m^2/mg and 9.89 nS m^2/mg, respectively. Between 1 GHz to 40 GHz the dependence of the permittivity on varying concentrations of insulin and bovine serum albumin was approximately linear, and had a slope of −0.08 mg/mL for bovine serum albumin and −0.12 mg/mL for insulin. The permittivity difference was normalized by the concentration and the unique permittivity of each protein was extracted.

1004-Pos
Cell and Droplet Sorting with Surface Acoustic Waves in Microfluidics
Thomas Franke[1], Achim Wixforth[2], David A. Weitz[1].
[1]Harvard University, Cambridge, MA, USA, [2]University Augsburg, Augsburg, Germany.
We describe a novel microfluidic cell sorter which operates in continuous flow at high sorting rates.
The device is based on a surface acoustic wave cell-sorting scheme and combines many advantages of fluorescence activated cell sorting (FACS) and droplet sorting in microfluidic channels (FADS).
It is fully integrated on a PDMS device, and allows fast electronic control of cell diversion.

We direct cells by acoustic streaming excited by a surface acoustic wave which deflects the fluid independently of the contrast in material properties of deflected objects and the continuous phase; thus the device underlying principle works without additional enhancement of the sorting by prior labelling of the cells with responsive markers such as magnetic or polarizable beads. Single cells are sorted directly from bulk media at rates as fast as several kHz without prior encapsulation into liquid droplet compartments as in traditional FACS. We have successfully directed HaCaT cells (human keratinocytes), fibroplasts from mice and MV3 melanoma cells. The low shear forces of this sorting method ensures that cells survive after sorting.

1005-Pos

High Frequency Chemical Stimulation of Living Cells

Jean-Christophe Galas[1], Mathieu Morel[2], Vasyl Shynkar[2], Maxime Dahan[2], **Vincent Studer[3].**
[1]ESPCI Laboratoire de Neurobiologie, PARIS, France, [2]ENS Laboratoire Kastler Brossel, PARIS, France, [3]CNRS Institut Francois Magendie, Bordeaux, France.

Fundamental and applied biomedical research increasingly relies on the quantitative analysis of the response of individual cells to controlled stimulations. The advent of microfluidics has revolutionized the methods to tailor the chemical environment of live cells, allowing stimulations to be applied with high spatio-temporal control and using minute amounts of reagents. It has been shown that Dictyostelium Discoideum cells (D.D.) can sense cAMP gradients on scales smaller than their size (a few tens of microns) and can adapt to very rapid concentration changes on sub second timescales. Classical methods to apply chemical stimulations, which are often based on the diffusion of solutes released by micropipettes, suffer from a poor spatial and temporal resolution and cannot discriminate the different timescales involved in the gradient sensing mechanism of D.D. Here we take advantage of a recently developed microfluidic toolbox based on microfluidic stickers made out of stiff polymers to stimulate adherent D.D. cells within microchannels. Microfluidic stickers allow for periodic chemical stimulation at the subcellular scale at frequencies up to 10Hz.We will present the dependence of the gradient sensing response as a function of both the temporal and spatial frequency. We will show how the microfluidic framework used in this study overcomes several experimental limitations and thus considerably extends previous investigations on the chemotaxis of D.D. by giving a new insight on previously proposed gradient sensing models.

1006-Pos

Ionflux: A Microfluidic Approach to Ensemble Recording and Block of Whole-Cell Current from Voltage-Gated Ion Channels

C. Ian Spencer, Nianzhen Li, Juliette Johnson, Qin Chen, Cristian Ionescu-Zanetti.
Fluxion Biosciences Inc, South San Francisco, CA, USA.

Automated patch clamp (APC) addresses a need for high throughput screening of chemical entities altering ion channel function. Systems that can produce pharmacologically relevant data rapidly and consistently find considerable utility in the pharmaceutical industry, but also increasingly in academic laboratories. Here we present data obtained with a novel APC platform utilizing a well-plate microfluidic design. Unlike existing devices, the IonFlux system incorporates no internal robotic liquid handling, and features continuous recording from cell ensembles during rapid solution switches with a bench-top footprint resembling a conventional plate reader.

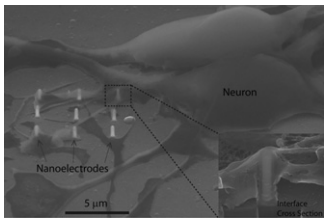
True whole cell voltage clamp was applied to linear arrays of up-to thirty cells in parallel, utilizing fully-featured 16 or 64 channel voltage-clamp amplifiers under computer control. Laminar flow of solutions in a microfluidic network delivered cells in suspension to the recording sites and enabled fast exchange of bathing solutions via an electro-pneumatic interface, on either 96 or 384 well-plate formats. Electrophysiological characterization was achieved for K_V 2.1 and hERG potassium channels, and examples of Na_V sodium channels. Our results show the voltage-dependence of these currents and their block by pharmacological agents. The recordings also demonstrate the potential for microfluidics-enabled, exceptionally fast superfusion and washout of candidate drugs. Incorporation of multiple experiments per well-plate enables many investigations to be performed in parallel, especially if multiple compounds are applied to each individual cell ensemble recording array. Data on recording success rates, throughput and assay reproducibility show that high throughput experiments can be performed with enhanced reliability.

1007-Pos

Nanoelectrodes for Neuron Recording and Stimulation

Chong Xie, Lindsey Hanson, Yi Cui, Bianxiao Cui.
Stanford University, Stanford, CA, USA.

Effective electronic neuron interface requires tight attachment between electronic devices and neuron cells. However, it is the nature of living cells to maintain an extracellular cleft between their membrane and the substrate which they adhere to, and the cleft contribute to most of the signal leakage. Nano-scale electrodes could enable tight attachment to cell membrane and thus form good electrical coupling with cultured neuron cells. We fabricate nano-electrodes by focused ion beam (FIB) Pt deposition. The electrodes are vertical aligned, and with diameter of 200 nm and height of 1 um. We cultured cortical neurons on the substrates with nanoelectrode arrays. Scanning electron microscopy (SEM) analysis shows that neuron cells engulf nano-electrodes readily. The tight engulfment provides good electrical coupling between neuron cells and nano-electrodes. We also examine action potential recording and stimulation with nano-electrode arrays. Image 1 shows SEM images of neuron nano-electrodes interface. The cross section view of the interface is milled by FIB.



1008-Pos

8-Parallel Bioparticle Sorter with a Multilayer PDMS Chip

Hirokazu Sugino[1], Kazuto Ozaki[2], Takahiro Arakawa[3], Yoshitaka Shirasaki[4], Yuki Nara[2], Shuichi Shoji[2], Takashi Funatsu[1].
[1]University of Tokyo, Tokyo, Japan, [2]Waseda University, Tokyo, Japan, [3]Tokyo Medical and Dental University, Tokyo, Japan, [4]RIKEN, Tokyo, Japan.

A major challenge in organelle proteomics is purifying the organelle of interest from cell lysate. We have been developing a fluorescence-activated microfluidic sorter for organelle purification. Flow switching mechanism of the sorter is based on the sol-gel transition of a thermoreversible gelation polymer (TGP). The TGP is liquid at low temperature and turns to a gel immediately upon heating. The TGP solution is mixed with bioparticles and introduced into a mirochannel that branches into waste and collection channels. When the fluorescence signal from a target particle is detected, the sol-gel transition of the TGP is induced at the entrance of the waste channel by heating with an infrared laser. This operation allows a fluorescent target to flow into the collection channel. In this study, eight parallel sorters were integrated in a microchip to achieve high-throughput sorting. The microchip consists of a glass cover plate and a PDMS block. The PDMS block has a multilayer structure with two inlets for sample and buffer solutions and two outlets for collection and waste solutions. Its structure makes it possible to form a sheath flow, i.e., sample-solution flow is focused by neighboring two buffer-solution flows in each sorter. We confirmed that sheath flows were formed in the chip. We demonstrated that fluorescent microspheres and *E. coli* cells were detected and sorted in each channel. A recovery ratio of 90% and a throughput of 20 particles/s were achieved. Future studies will allow us to integrate 16 or 32 channels into a single chip. The parallel sorter is a promising device for purifying large amount of bioparticles.

1009-Pos

Capture & Release of Single Cells on a Microfluidic Chip Via Conical Nanopores

Eric Hall[1], Peng Guo[2], Charles Martin[2], Richard N. Zare[1].
[1]Stanford University, Stanford, CA, USA, [2]Univeristy of Florida, Gainesville, FL, USA.

In modern biology, it is often assumed that populations of cells are composed primarily of average cells; cells that do not deviate significantly from an observational mean. This assumption is empirically convenient and until recently was necessary due to technological limitations. However, it is possible that ignorance of cellular individuality may lead one to draw incorrect conclusions, especially when the population under study is heterogeneous. Cells that exhibit significant deviation from the mean behavior can reveal important information which would be normally obscured by ensemble averaging techniques.

We have developed an array of microfluidic analytical techniques capable of studying the biochemistry of single cells [1,2,3]. Our current effort focuses on the development of a device which can capture a significantly large number of unicellular organisms resolved at the single-cell level. In our microchip, *Synechococcus* cells are captured via electroosmostic flow on a thin polycarbonate membrane that is populated by conical nanopores [4]. Cells can be efficiently captured and released via control of the flow's directionality. Applications of this chip, including those for high-throughput selective sorting and on-chip culturing, are being explored.

1. Wheeler, A. R., Throndset, W. R., Zare, R. N. et al. *Anal Chem* **75**, 3581-3586 (2003).
2. Wu, H., Wheeler, A. & Zare, R. N. *Proc Natl Acad Sci U S A* **101**, 12809-12813 (2004).
3. Huang, B., Wu, H., Zare, R. N. et al. *Science* **315**, 81-84 (2007).
4. Scopece, P., Ugo, P., Martin, C. et al. *Nanotech.* **17**, 3951-3956 (2006).