999-Pos
Inhomogeneous Morphology and Elasticity of Mouse Oocyte Zona Pellucida Pre- And Post-Fertilization
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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 poly-lysine 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

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Understanding the Stretching of DNA Molecules Confined in Nanofluidic Channels
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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
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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
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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-$K_{xx\text{-}yy}$' 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-$K_{0\text{-}25}$, behaved like the D2-based ICCR, showing channel inhibition upon dopamine application. Two $ß_2$-based ICCRs were successfully constructed, $ß_2$-$K_{62\text{-}25}$ and $ß_2$-$K_{73\text{-}25}$. 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-$K_{0\text{-}25}$, 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
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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
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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.