bond with the backbone, resulting in a cyclic conformation with a fixed alignment of the two carbonyls. The amidated form of glutamine has been studied at varying pH and temperature; data has been compared to that obtained for amidated alanine, amidated asparagine and non-amidated glutamine.

1595-Pos Board B439
Chemical Exchange 2DIR of Base Pair Opening Fluctuations in RNA Tetraloops: A Simulation Study
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Hydrogen bonds play an important role in RNA structure and dynamics. Fluctuations in base pair openings could be the starting point of unfolding processes or could indicate potential docking sites for ligands or proteins. The effects of hydrogen bond formation and breaking kinetics in RNA base pairs on the linear and coherent third order infrared spectra of small UUCG tetraloops in solution can be described by Markovian, not necessarily Gaussian, fluctuations. We have simulated these spectra using the stochastic Liouville equations. Slow fluctuations are described phenomenologically. Fast fluctuations are characterized by an N-state jump model for hydrogen bond configurations, where N depends on the specific tetraloop. Bases in the RNA strands that exhibit high levels of fluctuation are isotope labeled and the chemical exchange 2DIR spectra are calculated. The existence and evolution of the resultant cross peaks at different waiting times provides information on the coupling interactions between base pairs in the loops, which will be used to help characterize unfolding mechanisms of the RNA strands.

1596-Pos Board B440
Nitrile-Modified Nucleosides as a Probe of Local Nucleic Acid Environments
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The nitrile functional group has been extensively investigated as a probe in proteins but not as a probe in nucleosides. The solvent-induced vibrational frequency shift of the nitrile band of 5-cyano-2′-deoxyuridine was examined in THF-H_2O mixtures. The nitrile stretching frequency ($\nu_{C\equiv N}$) exhibited moderate solvent sensitivity, undergoing a 9.2 cm^{-1} blue shift from THF to H_2O, and varied linearly with temperature, exhibiting a 1.4 cm^{-1} red shift from 290 K to 340 K in H_2O. The $\nu_{C\equiv N}$ of 5-cyano-2′-deoxy-3′,5′-bis-O-(*t*-$BuPh_2Si$)-uridine underwent a 1.3 cm^{-1} blue shift when titrated with a base-pairing mimic, 2,6-diheptanamido-pyridine to yield an association constant of 90 M^{-1}. The corresponding $C^{15}N$ labeled nucleoside is currently under investigation by ^{15}N NMR to determine the utility of the $C^{15}N$ moiety as an NMR probe of the local environment of nucleic acids. Both the IR and NMR results will be presented complemented by density functional theory calculations.

1597-Pos Board B441
Multidimensional Optical Spectroscopy Of Proteins Out Of Thermal Equilibrium
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In recent years, nonlinear multidimensional optical spectroscopy has been used as a highly sensitive probe of molecular dynamics in the condensed phase. Multidimensional optical spectroscopy builds upon the methodology of two-dimensional nuclear magnetic resonance spectroscopy and applies the same principles to vibrational and electronic resonances such that these techniques may be used as an ultrafast probe of molecular dynamics. In particular, these techniques have been used to study the thermal unfolding of proteins following a nanosecond temperature jump. In this study, we examine the multidimensional optical spectra of several biological systems of interest out of thermal equilibrium by using molecular dynamics to develop snapshots of the systems and the SPECTRON software package to calculate the spectroscopic signals. In order to enhance conformational sampling, an artificial temperature is used; the exact correlation functions of the system contributing to the material response are recovered using an action-reweighting scheme based on a stochastic path-integral formalism. The calculated spectra provide information on the states sampled by the system during the course of thermal unfolding.

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1598-Pos Board B442
Spatially-resolved Analysis Of DNA Nanocomplex Self-assembly Enabled By Integrating Nanophotonics And Microfluidics
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Advances in genomics continue to fuel the development of future therapeutics that can target pathogenesis at the cellular and molecular level. Often functional only inside the cell, nucleic acid-based therapeutics require an efficient intracellular delivery system. One widely adopted approach is to complex DNA with a gene carrier to form nanocomplexes via electrostatic self-assembly, facilitating cellular uptake of DNA while protecting it against degradation. The challenge, however, lies in rational design of gene carriers, since premature dissociation or overly stable binding would be detrimental to the cellular uptake and therapeutic efficacy. Nanocomplexes synthesized by bulk mixing showed a diverse range of intracellular unpacking and trafficking behavior, which was attributed to the heterogeneity in size and stability of nanocomplexes. The heterogeneity of nanocomplexes resulting from bulk synthesis hinders the accurate assessment of the self-assembly kinetics and adds to the difficulty in correlating their physical properties to transfection efficiencies or bioactivities. We present a novel convergence of nanophotonics (i.e. QD-FRET) and microfluidics to characterize kinetic aspect of the nanocomplexes synthesis under laminar flow in real-time. QD-FRET provides a highly sensitive and quantitative indication of the onset of molecular interactions and throughout the process of nanocomplexes synthesis, whereas microfluidics offers a well-controlled microenvironment to spatially analyze the process with high temporal resolution (~milliseconds). For the model system of polymeric nanocomplexes, two distinct stages in the self-assembly process were captured by this analytic platform. The kinetic aspect of the self-assembly process obtained at the microscale would be particularly valuable for microreactor-based reactions which are relevant to many micro- and nano-scale applications. Further, customized nanocomplexes may be generated through proper design of microfludic devices, and the resulting QD-FRET polymeric DNA nanocomplexes could be readily applied for establishing structure-function relationships.

1599-Pos Board B443
Oligonucleotide Microarray Analysis with Single Molecule Sensitivity
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We present a microarray analysis platform, which enables detection of hybridized DNA sequences at the level of single molecules. The readout is performed on a high sensitivity chip scanner based on an fluorescence microscope. Capture sequences were printed on custom-made aldehyde-functionalized glass coverslips. The microarray performance was tested with a 60mer fluorescent oligonucleotide hybridized to its complementary sequence, immobilized on the biochip. The determined dynamic range of the platform reaches 4.7 orders of magnitude with a sensitivity of 1.3fM. Furthermore mRNA expression profiling experiments of tetracycline (un)treated HaCat cells were performed. For such competitive hybridization experiments only 5% reverse transcribed cDNA out of 5µg total RNA were hybridized, a hundredfold lower amount then used typically for commercial microarrays. Such wide range in detection sensitivity needs reliable methods for exact data quantification. At low concentration the signal of each spot and molecule brightness was quantified by counting the molecules, fitting them with a 2-dimensional Gaussian function. For high concentrations, the number of molecules per spot was inferred from the total signal per spot. Good correlation of the data with experiments on commercial microarrays using hundredfold higher sample amounts indicates the feasibility of this approach, which avoids application of error prone amplification methods.
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1600-Pos Board B444
Viscosity Measurement of Biological Fluids Using Optical Tweezer
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An optical tweezer (OT) has been widely used to study the mechanical properties of microscopic living biological systems like red blood cells. These studies are based on measurement of deformations caused by a force exerted directly or indirectly by an optical trap. The trap is usually pre-calibrated using Stokes viscous force of the suspension fluids for the biological system which is directly proportional to the viscosity of the fluids. Therefore, calibration of the trap depends on the viscosity of the fluid which depends on temperature. In this work, we have demonstrated that OT can be used to precisely measure the viscosity of biological fluids affected by temperature. Using a an infrared laser trap which is calibrated using a 3.1micron silica sphere suspended in a distilled deionized water and measuring the power as function of escape velocity, we have measured the viscosities of a newborn and unborn bovine serum with a different concentration of antibodies. Comparative analysis of these measurements with the measurements carried out by direct use of a viscometer have revealed a significant effect of increase in temperature resulting from the intense beam of the laser trap.

1601-Pos Board B445
Line Scanning Flow Measurements
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We are developing a new technique to measure flow of micron scale particles using laser scanning. This technique will also detect complex flow patterns, identify stationary particles and determine particle size. In this method, a laser beam is raster scanned over an area containing a flowing liquid. Particles in the liquid scatter the laser. Detailed information about the flow can be obtained from analyzing the fluctuations in this scattered radiation.
Detailed flow information, such as can be provided by this technique, is valuable in medical applications. Blood cells can serve as the particles that scatter lights and the laser scanning can be applied to surgically exposed blood vessels in a patient or in a animal model. The information available with this method can help study or monitor conditions such as sickle cell anemia in which abnormal blood cells do not move smoothly through blood vessels or become stuck. It can also be used to study the formation of atheromatous plaques. One factor in the creation of these plaques on artery walls is the accumulation of platelets and leukocytes. Understanding what prevents blood cells from flowing normally and what causes them to accumulate would be a significant improvement in our understanding of vascular disease.
This work was supported by the National Center for Research Resources of the National Institutes of Health (PHS 5 P41-RR003155).

1602-Pos Board B446
Towards Growth Cone Guidance On Silicon Chips By Capacitive Stimulation Of Voltage Dependent Ca2+ Channels
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Hybrid systems of neuronal networks and microelectronic chips can be used to elucidate network processes like learning and memory. Systematic experiments on network dynamics require a well defined topology of the synaptic connections. We want to control the directional outgrowth of neurites directly from the chip. Intracellular Ca2+ concentration $[Ca^{2+}]_i$ of growth cones is known to play a decisive role in neuronal outgrowth. By capacitive stimulation of voltage dependent Ca2+ channels (VDCCs) we want to manipulate $[Ca^{2+}]_i$ to steer growth cone guidance.
To show the feasibility of capacitive opening of VDCCs, we used HEK293 cells expressing L-type VDCC Cav1.2. The capacitive gating of Cav1.2 was studied under whole cell voltage clamp and current clamp conditions. We detected the Ca2+ influx by Fura-2 fluorescence microscopy. We found that the cells $[Ca^{2+}]_i$ was greatly enhanced by repetitive capacitive chip stimulation.
In a next set of experiments, we stimulated VDCCs in large, nonmotile growth cones of A-Cluster neurons from fresh water snail *Lymnea stagnalis*. We monitored growth cone $[Ca^{2+}]_i$ by Fura-2 fluorescence microscopy and found that repetitive capacitive stimulation induced profound changes in $[Ca^{2+}]_i$. Observation of growth cone morphology before, during and after repetitive stimulation revealed significant structural reorganisation that relates to growth cone collapse and repulsion.
Our results provide a first step towards capacitive control of growth cone guidance on silicon chips. Further experiments with smaller, motile growth cones have to be performed to achieve chip-controlled directional neurite outgrowth.

1603-Pos Board B447
A Novel Protein Array Using Microbeads Aligned In A Microfluidic Chip
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Protein array is a powerful means to investigate protein-protein interactions. Yet current protein arrays are not versatile due to their low sensitivity (>1ng/ml) and cost-ineffectiveness.
In this study, we have developed a sensitive and cost-effective protein array using a commercial fluorescence microscope. The protein array has aligned antibody-immobilized microbeads (5 μm in diameter) inside a polydimethylsiloxane (PDMS) microfluidic chip. The minimum concentration requied for fluorescence detection was determined to be several tenths of pM (about 1 pg/ml) using fluorescently-labeled glutathione-S-transferase (GST) to the protein array having α-GST antibody immobilized microbeads.
Firstly, we tried detecting a recombinant protein expressed in cultured cells. We extracted cytoplasmic components of PC12 cell expressing green fluoresent protein (GFP) and labeled them with amino group reactive fluorescent dye. The labeled product was applied to the protein array having α-GFP antibody microbeads, α-β-actin antibody microbeads as positive control and α-IgE antibody microbeads as negative control. Only α-GFP and α-β-actin antibody microbeads were fluorescent, demonstrating that the protein array is capable of detecting a target protein in cytoplasmic extract containing a large number of other proteins.
At present, to test its applicability to endogenous proteins, we are trying to detect expression levels of transcription factors, c-Jun and c-Fos, in Hela cell by the method mentioned above with their counterpart antibody microbeads.

1604-Pos Board B448
Evoking and Resolving Quantal Neurotransmitter Release on a Microchip
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A microchip that facilitates *in-vitro* electrical and electrochemical measurements of individual cells and cell clusters was fabricated using surface micromachining and thick film technologies. In the present study, the device was applied towards the detection of exocytotic events from electrically stimulated rat pheochromocytoma (PC12) cells. Using device microfluidics, cells were positioned in a recording chamber over a 5 μm × 10 μm gold working electrode (WE). Channel dimensions (10 μm deep × 10 μm wide) ensured a tight fit for the ~12 μm diameter PC12 cells in the chamber resulting in direct contact of the cells with the WE. This proximity allowed for quantal resolution of catecholamine release events from the cells and corresponding analysis of release kinetics and quantal size. Cells were stimulated through the application of sinusoidal voltage waveforms across axially-positioned, extracellular electrodes. In this manner, patterned extracellular gradients were generated across the cell thereby resulting in membrane depolarization. To facilitate interpretation of the stimulating electric field in relation to the cell and subsequent dopamine release, quasi-static electromagnetic FEM models were generated using COMSOL Multiphysics software. Upon depolarization, simultaneous chronoamperometric recordings at the WE confirmed stimulus-triggered dopamine release from the cells with a small subset of cells exhibiting release that modulated with the depolarizing cycle of the sinusoidal stimulus. It is anticipated that such a chip could provide a semi-automated alternative to the conventional, labor-intensive carbon fiber electrode (CFE) approach to neurotransmitter measurement.
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1605-Pos Board B449
Silicon Chip Patch-clamp Electrodes Integrated With Pdms Microfluidics
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We have developed an integrated planar patch-clamp system for the acquisition of ion channel activity from single cells. The system consists of a pore within a suspended silicon oxide membrane integrated with PDMS microfluidics. The silicon electrodes have enabled the achievement of gigaohm seals in high yield and the electrical nature of the cell/wafer seal has been characterized for several pore geometries. The PDMS microfluidics allow the placement of a single cell directly over the silicon pore hydrodynamically within PDMS microfluidic channels, without user input. Furthermore, the microfluidic channels permit the use of low solution volume and very rapid extracellular and intracellular solution exchange. This device enables a real-time, *multi-parameter* analysis on high-density arrays of single cells in distinct physiological environments.

1606-Pos Board B450
Automated Reactor For Extraction And Manipulation Of Sub-Megabase Fragments Of Genomic DNA With Flow-Focusing
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