by the cellulases. One of the important enzymes in the process is β-glucosidase (BGL), which catalyzes the hydrolysis of cellobiose to glucose. In the present study, we aim to obtain BGL mutants with improved hydrolytic activity by molecular evolution. To screen many potential mutants easily and precisely, we have developed the enzymatic assay for β-glucosidase using a microchamber array chip.
BGL1B from the basidiomycete Phanerochaete chrysosporium was used as the target enzyme. The enzyme was overexpressed in E. coli, and purified to homogeneity. The purified enzyme was confined with fluorogenic substrate (TokyoGreen β-Glu) in microchambers with a volume of 50 fL. The microchamber array chips were fabricated by micromolding processes with polydimethylsiloxane (PDMS) polymer. The enzyme can produce a large number of fluorescent molecules (TokyoGreen) by hydrolyzing TokyoGreen β-Glu, leading to amplification of the fluorescent signal in a microchamber. When BGL1B solution was diluted to a few molecules in each chamber, fluorescence signals from chambers heterogeneously increased with time. The distribution of fluorescent increment rates showed quantized and evenly spaced peaks. The peaks were considered to be attributed to 0, 1, 2 and 3 BGL1B molecules per chamber. The result indicated that the microchamber array chip-based assay system is valuable for evaluating the BGL activity quantitatively. Now, we are trying to apply this assay system to screening of BGL1B mutants.

936-Pos Board B722
Manipulation of Actin Networks *In-Vitro* by Engineering Interfacial Roughness
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Engineering biological complexity into *in-vitro* assays of purified components requires an understanding of self-assembly on the nano-scale. Using a controllable nanoengineered surface that alters the dynamics of filamentous actin adhesion, we studied the tunability of biomolecular surface attachment. By grafting nanoparticles ranging from 12 to 85 nm diameter to a poly(styrene-random-acrylic acid) copolymer film at different densities and attachment times, the size and spacing of substrate textural features were varied. The nature of f-actin attachment was characterized by TIRF microscopy. On selectively generated monodisperse and polydisperse features of varying size and areal density, f-actin binding was characterized as immobilized, side-on wobbly, or end-on attached. The varied types of interactions between actin and the substrate are explained by an energetic penalty associated with actin bending around surface topographic features, given by the worm-like chain model, in conjunction with attractive forces and experimentally determined surface parameters. Comparing the repulsive term due to bending with the electrostatic attraction of actin to the surface provides insight into how the three types of actin binding result on textured surfaces which are chemically very similar. Stepping velocities of single fluorescent labeled myosin V molecules were compared on actin filaments bound to the surface with features of varied roughness. Immobilization of actin filaments on the nano-featured surfaces moderately reduces velocity, while motility on all surfaces indicates that polymer-nanoparticle composite surfaces are flexible candidates for the fabrication of biomolecular devices.
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937-Pos Board B723
Inititiation, Flow Rate, and Routing of Capillary-Driven Flow of Liquid Moving through Microchannels on a Slipchip
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This study is devoted to controlling the initiation and flow rate of spontaneous liquid-liquid flow passing through microfluidic channels in response to capillary action on a non-wetting droplet. Aqueous droplets were generated on a hydrophobic SlipChip in a shallow channel, and then a stepwise change in capillary force was induced by connecting the shallow channel to a deeper channel filled with immiscible oil. A model to predict the rate of spontaneous flow was developed based on the balance of net capillary pressure with viscous flow resistance; the inputs to the model were the liquid-liquid surface tension, advancing and receding contact angles at the three-phase aqueous-oil-surface interface, and the geometry of the device. The effects of contact angle hysteresis, presence or absence of a lubricating oil layer, and adsorption of surfactants at liquid-liquid or liquid-solid interfaces were quantified. Two different regimes of flow were studied. Faster (mm/s) flow rates were obtained when oil being displaced by the aqueous could escape through connected channels, and slower (μm/s) flow rates were obtained when displaced oil could escaped only through a μm-scale gap between the plates of the SlipChip (“dead-end flow”). Both diluted salt solutions and complex biological media such as human blood plasma were found to flow using this approach, and we anticipate it being useful in the future for control of flow in microfluidic designs that do not require external power, valves, or pumps. Approaches based on initiation of spontaneous flow would be useful for design and operation of the SlipChip platform as well as for other droplet-based and plug-based microfluidic devices.

938-Pos Board B724
Magnetic and Metal Binding Structural Analysis of Mn,Zn-Metallothionein-Green Fluorescence Fusion Protein
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Metallothionein-green fluorescence fusion protein (MT-GFP) is a zinc binding protein, which binds to seven divalent transition metal ions through its 20 conserved cysteines and forms two metal binding clusters with Zinc-Blende structure. In this study, we substituted Mn^{2+} for Zn^{2+} at M_3, M_4 metal binding sites in the b-domain of MT-GFP. We found this Mn, Zn binding protein exhibited ferromagnetic properties from 10K to 300K by SQUID measurement. By micro-Raman spectroscopy analysis, the Zn-S and Mn-S bending modes can observed clearly at 288 cm^{-1} and 355 cm^{-1}, respectively. These indicate that the Zn^{2+} and Mn^{2+} are bound with Cys residues of MT-GFP. Extended X-ray absorption fine structure (EXAFS) analysis also indicated Mn^{2+} binding to MT-GFP via the Mn-S bond of Cys.

939-Pos Board B725
Taxol-Conjugated Pamam Dendrimers Utilize Three Modes of Action on Microtubule Structure
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Paclitaxel (Taxol) is a cancer drug that causes cell death by stabilizing microtubules and consequently arresting cell division. Previously, the cytotoxicity of taxol-conjugated PAMAM dendrimers was demonstrated in cancer cells. The exact mode(s) of action responsible for the potent cytotoxicity of these dendrimers were not examined but the literature provides uncertainty that the taxol could be released from the dendrimer carrier after cellular entry. Accordingly, we asked whether the taxol-dendrimer conjugate itself is able to bind microtubules. To address this question, we investigated the effect of these conjugates on microtubules in vitro using total internal reflection fluorescence microscopy (TIRFM) and transmission electron microscopy (TEM). We find that the taxol-dendrimer conjugate affects microtubule structure in two ways: (1) the conjugate can bind tubulin during tubulin polymerization and stabilize it into a tubular structure and (2) the conjugate can bundle microtubules in a manner that is not dependent on taxol, but dendrimer electrostatics. Both of these modes of action would arrest cell division and consequently kill the cell. This is the first time that the binding of taxol-conjugated dendrimers to microtubules has been demonstrated in vitro. Furthermore, our results provide both mechanistic insights into the cytotoxicity of the previously characterized taxol-conjugated PAMAM dendrimers and additional evidence for the potential of these and similar conjugates as cancer therapeutics.

940-Pos Board B726
A Microfluidic-Based Tyrosine Kinase and Phosphatase Assay
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Protein interactions describe a significant part of a biochemical pathway. However, a real biochemical pathway has other dimensions as well. For example, enzymatic reactions or post-translational modifications (PTMs). The most common PTM known is phosphorylation. This PTM facilitates many cellular functions such as signal transduction, cell communication, cell cycle, differentiation, protein synthesis and more. Overall, PTMs are an integral part of all biochemical pathways. Therefore, in order to recapitulate a biochemical pathway in vitro, the role of PTMs must be addressed. We have decided to