developed protocols to use these surface coated quantum dots for intravitral imaging in rodents and we are able to successfully acquire in vivo images of the kidney and perform kinetic measurements. Here, we summarize and discuss our results of the PFE property and in vivo imaging and characterization of these quantum dots. This work was supported by NIH DK077051 research award to W. Yu on in vivo kidney imaging.

177-Pos Board B56
Time-lapse Imaging of Individual BKCa Channels in Live Cells Using Site-specific Labeling of Quantum Dots

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Although the proper localization of specific ion channels at certain regions of cell membrane is essential for their cellular functions, it is a great challenge to visualize and to trace individual channel proteins in live cells. We utilized quantum dots (QDs) to label the large-conductance Ca²⁺-activated K⁺ channels (BKCa channels) and monitored their movement in real-time. A site-specific biotinylation was achieved by genetically inserting the ‘acceptor peptide’ sequence at the extracellular N-terminus of the channel and by co-expressing the channel with the E. coli biotin-ligase modified to target into endoplasmic reticulum. After brief incubation of streptavidin-conjugated QDs, strong cell surface labeling of QDs was detected in both COS7 cell and cultured hippocampal pyramidal neurons. By tracking the labeled QDs using time-lapse imaging, we were able to monitor single BKCa channels with high resolution in live cells. In addition, two-color pulse-chase labeling allowed us to observe the channel trafficking to cell surface membrane de novo and their redistribution in real-time. Using the time-lapse imaging of QD-labeled channel protein as an assay system, we were able to show the differential roles of cytoskeletons and their redistribu-

178-Pos Board B57
Non-Invasive Pyrenebutyrate-mediated Delivery of Quantum Dots to the Cytosol of Living Cells

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Quantum dots are nanometer-diameter fluorescent probes made of semiconductor materials. Compared to organic fluorophores, quantum dots are highly photo-stable and very bright making them optimal for live cell imaging. A cationic peptide on the quantum dot creating a pyrenebutyrate-quantum dot complex is trapped in vesicles and is unable to access cytosolic components. Pyrenebutyrate, an aromatic, hydrophobic molecule, interacts with the cationic peptide on the quantum dot creating a pyrenebutyrate-quantum dot complex that can bypass the endocytic pathway. The transport of the quantum dot across the plasma membrane is not inhibited at 4°C, lacks colocalization with endocytic markers, and has very little active motion within the cell. This suggests that the mechanism of transport is not endocytosis, but instead direct transport across the plasma membrane. A cell viability assay done with trypan blue determined that incubation with the highest concentration of pyrenebutyrate did not show any harmful effects. By bypassing the endocytic pathway this allows for targeting of cytosolic proteins difficult to label in live cells.

Molecular Mechanics & Force Spectroscopy

181-Pos Board B60
Nucleosome Stacking Defines The Structural And Mechanical Properties Of Chromatin Fibers

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In eukaryotic cells, genomic DNA, and core histones form dense 30 nm chromatin fibers. This compaction is driven by stacking of nucleosomes and has been implicated to regulate gene expression. We investigated the mechanical properties of reconstituted chromatin fibers containing 25 repeats of the 601 nucleosome-positioning element. The force-extension curves of these chromatin fibers were measured with magnetic tweezers. The fibers are well characterized by three springs in series: a worm like chain (WLC) of the flanking DNA, a Hookeian spring of the 30 nm fiber, and a WLC of the ruptured fiber. Using this analysis we unambiguously demonstrated that nucleosome stacking drives a fiber with 197 bp repeat length into a solenoid helix. The model quantities force dependent and Mg²⁺ dependent nucleosome unstacking, resolves the structural heterogeneity of 30 nm fiber folding, and reveals fivfold higher nucleosome-nucleosome interaction energy than reported before (Cui and Bustamante, 2000). This provides a complete structural and mechanical description of the high order folding of chromatin fibers.