membrane of specific types of carcinoma cells. Specifically, the over-expression in certain epithelial carcinoma cells can be as high as a thousand fold and thus FR provides an important possible target in efficient drug therapy design. In this study, we are probing the dynamics and interactions of folate/FR receptor complexes in the plasma membrane and through each part of its endocytotic cycle at the single molecule level. We are employing single molecule fluorescence microscopy to track individual fluorophore-labeled folate molecules bound to cell surface FR. We found an average diffusion constant of D = 2e-9 cm^2/s on live human KB carcinoma cells when imaged at 30 fps. Trajectories of individual particles showed temporary stopping or confined motion. This anomalous diffusive behavior was quantified against Monte Carlo simulations of randomly diffusing particles. The frequency and duration of confinement was compared as the overall concentration of folate was incrementally increased from 1 fM to the physiological level of 1nM . The effect on the frequency and duration of single FR confinement due to cholesterol depletion, actin stabilization and actin depolymerization will be presented.

1440-Pos Board B284
The Role of Cluster Size and Protein Spatial Pattern in the Immunological Synapse
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During antigen recognition by T cells, signaling molecules on the T cell engage ligands on the antigen-presenting cell and organize into spatially distinctive patterns collectively known as the immunological synapse (IS). The spatial arrangement of proteins into well-defined zones within the IS is known to regulate T cell activation and signal transduction. The mechanisms by which this complex organization arises remain unclear. Here we alter the clustering state of the T cell costimulatory molecule, LFA-1, either by direct antibody crosslinking or by crosslinking its ligand, ICAM-1, displayed on the supported bilayer. Changes in receptor clustering lead to progressively more central localization of LFA-1 until it colocalizes with T cell receptors (TCR) at the center. The number of LFA-1 molecules within the resulting clusters is obtained by fluorescence correlation spectroscopy. Our results demonstrate that cluster size is a critical parameter in determining protein spatial positioning in the IS. We discuss a sorting mechanism, based on frictional coupling to the cytoskeleton, which is consistent with these observations and is, in principle, extendable to all cell surface proteins in the synapse. Furthermore, by presenting patterns of immobilized ICAM-1 within a fluid bilayer displaying the TCR ligand, peptide-loaded MHC, we investigate the importance of LFA-1 ring formation to T cell function and signaling.

1441-Pos Board B285
Multispecies Single Molecule Imaging With Quantum Dots
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Quantum dots (Qdots) are fluorescent nanoparticles that have far superior signal intensity and signal stability compared to more conventional fluorescent molecules. We find that imaging with Qdots can easily be extended to the simultaneous visualization of up to four different molecular species at single molecule sensitivity and millisecond time integration. We find that this technique can easily be adapted towards studying the spatial and temporal nano-organization of various combinations of lipids and proteins in the cellular plasma membrane.

Intracellular Communication & Gap Junctions

1442-Pos Board B286
Structural And Functional Significance Of The N-terminus Of Cx26 Gap Junction Channels
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Investigations of gap junction channels have used different and often complementary experimental approaches to elucidate the gating mechanism of these intercellular conduits. However, no consensus gating model explaining the different phenomena of voltage and chemical gating has been formulated that is consistent with all mutagenesis, dye permeation, electrophysiology and electron microscopy results.
We made several N-terminal deletion constructs to assess channel structure/function. Shorter deletions such as del2-7 and del2-8 formed gap junctions when expressed in mammalian cells while longer constructs (del2-10 and 2-11) were either much less efficient or failed to form gap junctions. For our structure analysis, a full length connexin26 (Cx26M34A) and an N-terminal deletion mutant (Cx26M34Adel2-7) were over-expressed using Sf9 insect cells. Purified proteins were reconstituted into the lipid bilayers that formed 2D crystals. The 3D maps at 10Å resolution revealed that crystals obtained from both constructs were composed of three lipid bilayers with the channels forming a $p22_12_1$ lattice. The structure of Cx26M34A clearly showed a prominent density we refer to as a ''plug'', which resides in each hemichannel pore and contacts the innermost helices of surrounding subunits at the bottom of the vestibule (Oshima *et al.*, 2007). The 3D structure of Cx26M34Adel2-7 contained a reduced plug and a partially reduced density in the cytoplasmic domain that bridges the adjacent four helix bundles, suggesting that the N-terminus of Cx26 has an important role in forming a plug. Cx26M34Adel2-7 exhibited no electrical functionality. Cx26M34A channels showed little or no dye transfer and a dramatically reduced conductance, although the voltage gating characteristics of the residual conductance were normal. Physical blockage may be one of the gating mechanism of Cx26 channels, however, this may represent only one of multiple gating configurations.

1443-Pos Board B287
Effects Of Induced Post-ischemic Phosphorylation On Action Potential Propagation In Mouse Neonatal Cardiomyocytes
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Intracellular events triggered by protein phosphorylation and kinase translocation have been reported to help preventing larger cardiac tissue damage. Our objective was to determine if immediate changes in phosphorylation post-infarction, resembling clinical treatments could affect conduction velocity and further cardiac protection.
Cardiomyocytes from 0-2 day old mice pups were grown on 64 Micro-Electrode Arrays (MEAs; MES, Germany). The MEAs recorded action potential (AP) propagation produced by neonatal cardiomyocytes isolated and culture for 2 days. Myocytes were subjected to ischemia by placing a 13mm glass round cover-slip over the preparation for 45 minutes and recordings were made during and after cover-slip removal. After the ischemic event, random groups were treated with 300nM TPA to induce phosphorylation for one hour. TPA was removed and both groups were incubated for 24 hours. Following incubation cells were subjected to another ischemic event. Recordings were done after the ischemic event and again thirty minutes later. Conduction velocity (CV) was averaged from all electrodes.
Phosphorylation and kinase translocation has been known to protect cardiocytes during ischemic preconditioning. We now present conditions where phosphorylation can actually be detrimental if induced after an ischemic event. At the 24 hour point, the control group had recovered AP CV by 99 ± 2% (mean ± SE) compared to the TPA treated group which recovered to 56 ± 8%. The TPA treated group then showed only an average of 14 ± 7% decrease in AP CV and AP amplitude upon administering the second ischemic event whereas the control group AP CV decreased by an average of 55% ± 9. When induced early after an ischemic event, phosphorylation results protective to maintain CV but appears to become detrimental for cellular survival.

1444-Pos Board B288
Different Types of Cell-to-Cell Connections Mediated By Nanotubular Structures
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Communication between cells is crucial for proper functioning of multi-cellular organisms. The recently discovered membranous tubes, named tunnelling nanotubes (TNTs), that directly bridge neighbouring cells, may offer a very specific and effective way of intercellular transport and communication. Our experiments on RT4 and T24 urothelial cell lines show that TNTs can be