2039-Pos
Probing the Interaction between a Nanopipette and a Soft Surface Using Scanning Ion Conductance Microscopy (SICM)
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Imaging cell topography to high spatial and temporal resolution is key for many areas of biological research. SICM, based on the scanned nanopipette, is a technique ideally suited to this and works by regulation of the ion current flowing through the tip opening.1 SICM is a noncontact method and works under physiological conditions making it an excellent method for high resolution imaging of cells. Previously this technique has been used to image numerous cell types2-5.
We have performed a detailed study of the small forces that are exerted on model oil droplet surfaces of known surface tension by the tip of a nanopipette. By varying salt concentration and applied voltage we can quantify the force. This force under our normal SICM imaging conditions is found to be very small but increases as the pipette approaches the surface. This allows non-contact cell imaging at high resolution while applying small forces of set magnitude, and has been used to image the underlying cytoskeleton of Osteoblast and Stem Cells.
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2040-Pos
Surface Charge Mapping Based on Scanning Ion Conductance Microscopy
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Scanning Ion Conductance Microscopy (SICM) is capable of high resolution, non-contact topographic imaging of live biological cells under physiological conditions(1, 2). The technique uses the ion current flowing through a nanopipette as a feedback signal to control the pipette probe over the surface. SICM has successfully been combined with a number of other techniques to study live cells. For example, SICM topographic imaging has been performed simultaneously with both patch clamping(3) and confocal microscopy(4). The integration of a surface charge mapping technique with SICM imaging is currently being developed.
The charge mapping technique has successfully been used to detect charge arranged in three-dimensions, such as in cationic and anionic polymer brushes. The next stage is to optimise the detection of two-dimensional charge distributions. The technique has the potential for efficient and reliable characterisation of surface charge throughout a biological membrane. Knowledge of membrane surface charge is important because membrane electrostatics affect the conformation and function of many biomolecules and also play a role in intracellular and intercellular recognition and transport(5).
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2041-Pos
Optimizing Protein-Based Optical Voltage Sensitive Probes: A Systematic Study
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Replacement of the phosphatase domain of the *Ciona intestinalis* voltage sensitive phosphatase (CiVSP) with fluorescence protein (FP) FRET pairs generates chimeric proteins that respond to voltage fluctuations with changes in FRET efficiency. VSFP 2.1 and Mermaid represent two different CiVSP-based voltage probes. We have developed a single FP-based, CiVSP-based probe (pKF215). These probes have different FPs and linker domains. As these constructs represent the most promising probes developed to date, we have undertaken a structure/function analysis of these constructs by: i) altering the inserted FPs and ii) replacing the VSD with species orthologs. The goal is to produce probes with a larger and faster ΔF/F response. We have created a matrix of 33 different constructs replacing the FPs of the original constructs with a range of red-shifted FPs (mCherry, mStrawberry, mTangerine, mOrange, tdTomato and TagRFP). We have also replaced the inserts with the two currently most viable FRET pairs (CFP/YFP and the Mermaid FRET pair). Finally we have chosen three other FPs which we have previously identified as 'modulatable' (mCerulean, PHluorin and eGFP). As the S1 to S4 domain of CiVSP has produced the most successful fluorescent voltage sensing constructs, we sought orthologs of Ciona VSD that might perform better. We identified VSPs orthologs from a variety of different species. We cloned the VSP orthologs from chicken, mouse, human, *Xenopus laevis*, *Xenopus tropicalis*, and zebra fish. To test these VSDs, we have created VSFP 2.1-like constructs from these cDNAs by inserting the CFP/YFP FRET pairs at homologous positions in the orthologous sequence. The constructs are being tested for membrane expression and voltage-dependent fluorescence changes in HEK293 cells.

2042-Pos
Solvatochromic Polarity-Sensitive Probes for Live Cell Imaging
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Polarity-dependent fluorescent probes are attracting increasing interest for high-resolution cell imaging. A notable example is that of solvatochromic dyes located in a domain where polarity changes occurring upon binding lead to the enhancement of the fluorescence signal. This can yield a very sensitive detection of molecular recognition events that is applicable even in the case of non-overexpressed proteins.[1] We shall present a toolbox of new solvatochromic coumarin derivatives that are suitable for *in vivo* imaging applications.
Following a preliminary screening by computational methods of a set of candidate-structures selected on the basis of their spectral properties, we designed a synthetic protocol allowing a broad range of substitution patterns. We shall present experimental data on several probes showing excellent fluorescence quantum yields (up to 0.95), high molar-extinction coefficients (up to 46,000 $M^{-1}cm^{-1}$), and large Stokes shifts. Notably, our molecules display marked solvatochromism: they are virtually non emissive in water, but intensely fluorescent in less polar media (we shall show up to 780-fold fluorescence enhancement). We shall also report on probe suitability for *in vivo* experiments. When tested on cultured cells, these coumarins proved not harmful and their photophysical properties were unchanged compared to data in solution. Owing to both their strong solvatochromic properties and their lipophilic character, the coumarins fluoresce only in the most lipophilic environments of the cell. In particular, colocalization experiments with organelle markers indicate that our coumarins locate in ER, membranes and lysosomes, depending on their chemical structure. Finally, one compound shows monoexponential decay of fluorescence with a lifetime which is linearly dependent on solvent polarity. We shall discuss how this feature promotes its use as ratiometric indicator of cell polarity at the nanoscale level.
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2043-Pos
Determination of Stoichiometry and Geometry of G Protein Coupled Receptor Homo-Oligomers in Living Cells Using Spectrally Resolved FRET
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Förster Resonance Energy Transfer (FRET) between donor and acceptor molecules is widely used for detection of molecular interactions. When donor and acceptor tags are fused to proteins of interest, FRET may be used to probe whether the tagged proteins form functional complexes in living cells. Typically, the cell under study is scanned at several times in order to accumulate enough spectral information to determine the FRET efficiency for each region of interest within the cell. However, diffusion as well as biochemical reactions may cause the molecular make-up of the regions of interest to change during the course of data acquisition. For a long time, this has dramatically limited the information content of FRET imaging. Advances in theory and optical instrumentation recently lead to the development of a FRET technique that avoids the problems caused by molecular diffusion and enabled us to determine the stoichiometry, structure, and localization in living cells of membrane protein complexes (Raicu et al., Nature Photonics, **3**, 2009). This presentation will review the results obtained from our recent studies of oligomeric complexes of some G protein-coupled receptors (GPCRs) in vivo, both, in the presence and in the absence of natural as well synthetic ligands.