understand the functional integration of these components. Our group have pioneered the development of an array of new and powerful biophysical tools based on Scanning Ion Conductance Microscopy (SICM) that allow quantitative measurements and non-invasive functional imaging of the surface of living cells down to the level of single protein molecules. Scanning ion conductance microscopy and a battery of associated innovative methods are unique among current imaging techniques, not only in spatial and temporal resolution of living cells, but also in the rich combination of imaging with other functional interrogation methods [1-3]. Recently we develop a novel class of nanoprobes based on double-barrel nanopipettes. One barrel is selectively functionalized with carbon and act as a nanoelectrode while the other one is used for ion conductance microscopy. These nanoprobes allowed simultaneous electrochemical and ion conductance imaging [4] and functional mapping of the electrochemical activity of the surface of living cells [5]. Current work in our group is directed to the use of these nanoprobes for intracellular sensing. We will present recent data demonstrating their ability to measure intracellular molecules in real time both in adherent cells and in tissue.
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4030-Pos Board B758
RNA Isolation from Single Living Cells using AFM
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Many protein-RNA complexes exist within cells and have various functions, including shuttling mRNA from the site of transcription to specific cellular regions. Often, these protein-RNA complexes consist of multiple mRNAs of which some are known and others remain unknown. The traditional method used to isolate protein-mRNA complexes is immunoprecipitation (IP). IP involves lysing a population of cells and isolating the protein-mRNA complexes using antibodies specific to the protein to "pulldown" the corresponding protein-mRNA complex of interest. We demonstrate the use of AFM as a complementary analysis tool for studying protein-RNA complexes in single living cells. While IP homogenizes the composition of protein-mRNA complexes from the cellular population under investigation and represents an "average", the AFM can interrogate not only a single cell but also subcellular regions of a single live cell, allowing stratification of protein-mRNA complexes from different regions of the cell. The result is the ability to spatially discriminate different populations of mRNAs that may be carried by the shuttling protein. To demonstrate extraction of a protein-mRNA complex, we selected the shuttling protein, Zipcode Binding Protein 1 (ZBP1) and its known associated mRNA, beta-actin, as a model system. Rat fibroblasts expressing a fusion protein, ZBP1-mCherry, were visualized using the fluorescent microscope of the AFM and then the cells of interest were punctured with an AFM tip conjugated with antibodies to ZBP1. Subsequently, the AFM tip was collected and analyzed for beta-actin mRNA using RT-PCR. BioAnalyzer results confirmed the extraction of beta-actin mRNA. This work shows that the AFM can be used as a tool to extract protein-RNA complexes from different regions of single living cells, potentially expanding the cell biologist's toolset.

4031-Pos Board B759
The Effects of Marine Bacteria on Barite Growth and Morphology
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Several studies have shown that bacteria can influence calcite ($CaCO_3$) crystal growth, although little is known about the effects of bacteria on barite ($BaSO_4$). Calcite and barite are found in comparable environments- namely under oceanic or sedimentary conditions. The purpose of this investigation is to determine the effects of a marine gram-negative bacterial strain on the growth and morphology of barite ($BaSO_4$). Using an atomic and lateral force microscopy, we established the typical crystal growth pattern and step velocity in supersaturated $BaSO_{4(aq)}$ solution. We then introduced the bacteria to the barite system and monitored changes in barite topography, friction, and step velocity. Preliminary data suggest a determinable alteration in the growth and morphology of the crystal when in the presence of the bacteria, leading us to infer that bacteria and sedimentary crystals discernibly affect one another. Future studies will connect these results to current discrepancies in oceanic mineral concentrations.

4032-Pos Board B760
Study Sub-Membrane Structure and Corresponding Functions of Conductive Bacteria Cable by SPMs
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As a kind of surface characterization tool, traditional scanning probe microscopy (SPM), especially atomic force microscopy (AFM), is hard to explore the structures underneath sample surface and correlate them with the corresponding functions. With the development of advanced AFMs, this obstacle has been overcome gradually, for example, with the application of quantitative dynamic AFM mapping, the hollow helical amyloid self-assembly fibrils have been identified;[1] and the protein structure flexibility on inner/outer sides of membrane is also possible to determine[2]. However, distinguishing the sub-surface features and corresponding function of macro-size biological samples, such as bacteria and cells, is still challenging to SPM. In this presentation, we combined quantitative dynamic AFM, AFM based manipulation, electrostatics force microscopy and scanning ion conductance microscopy to illustrate the inside membrane feature of recently identified conductive bacteria cable[3]. Basing on the SPM results, we proposed the model to explain the reason of the bacterial function to transport electrons over centimeter distance along cable direction. On one hand, this work will help biologists to understand the bacterial cable and promote the future application in nano-conductive cable field; on the other hand, it will inspire the further applications of SPM to beyond surface limitation on macro-size bio-systems.
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4033-Pos Board B761
Structural Dynamics of Proteasome: AFM Perspective
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Conformational transitions and allosteric signaling are the major contributors to catalytic activity of enzymes. The proteasome is a giant, multisubunit enzyme constituting a prime example of highly dynamic and allosterically regulated structure. This essential protease of the ubiquitin-proteasome pathway is a target of successful anti-cancer drugs. To explore alternative means of proteasome regulation, a better understanding of its mechanism is highly desired. However, its size and complexity puts it beyond the reach of most of the methods probing the global structural dynamics. Here we report results of testing dynamics of a catalytic core of proteasome with atomic force microscopy (AFM). We focused on the mechanism of elements comprising the gate controlling access to a channel that leads to a catalytic chamber internalized within a tube shaped particle. We identified conformational diversity within a single molecule of the proteasome using the oscillation mode AFM in liquid and followed the allosteric routing between the gate and active centers. We established that a gate opening and closing cycle represent activity readout of the catalytic centers. Moreover, we found that reciprocally the gate elements affect kinetics of catalysis. Now, we extended these studies by applying the high speed AFM to investigate the mechanism underpinning the very process of gate movements and position shifts of surrounding subunits. For the first time, it was possible to directly follow a complete cycle of the working gate in the live 20S proteasome with a high temporal resolution. The highest speeds of a tip reached 163 nm/ms enabled to examine changes in the gate structure occurring within 10 microseconds. Partition of the gate conformers and analysis of single molecule gate cycles detected with diverse tip speeds brings a promise to reveal details of proteasome structural dynamics not available with any other structural approach.