Biopolymers in Vivo

3706-Pos Board B434
A Fast, High-Throughput, and Highly Sensitive Analysis of Bacterial Cell Walls using Ultra Performance Liquid Chromatography
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The bacterial cell wall, also known as the murein sacculus, is composed of glycan chains crosslinked by short peptides. The maintenance of the integrity of the sacculus is complex, as it is involved in both shape determination and growth in virtually all bacteria. We aim to gain a quantitative understanding of the relationship between peptidoglycan architecture, morphogenesis, and pathogenesis. We have used Ultra-hydro Pressure Liquid Chromatography (UPLC), a new highly sensitive HPLC analysis, to measure the average length of the glycan strands from which the sacculus is made, the degree of cross-linking between those strands, and muropeptide identity of rod-shaped bacteria. Sacculi from Escherichia coli laboratory strains were analyzed and compared to ureapathogenic E. coli, and E. coli treated with A22, a small molecule drug that depolymerizes the actin homolog MreB and leads to a round morphology. We have also quantified muropeptides from different strains of Pseudomonas aeruginosa and Vibrio cholera to further correlate cell wall composition with pathogenesis. Preliminary findings indicate no significant change in peptidoglycan composition between strains from the same bacterium, although bacteria differ from each other in relative amounts of muropeptides, glycan strand lengths, and crosslinking. Neither pathogenicity nor changes in cell shape result in any significant differences in cell wall quantities. We are in the process of identifying unique muropeptides that develop with shape change and in clinical isolates using mass spectrometry. UPLC is a quick, reliable alternative to HPLC analysis of peptidoglycan, and provides a robust method for precise quantification of the complex chemical composition of the bacterial cell wall.

3707-Pos Board B435
A Conformational Landscape for Alginate Secretion Across the Outer Membrane of Pseudomonas Aeruginosa
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The exopolysaccharide alginate is a key component of the biofilms produced by Pseudomonas aeruginosa, a major pathogen that contributes to the demise of cystic fibrosis patients. Biofilm formation is a major factor in determining the stability and persistence of these bacteria. Alginate is synthesized within the cell and is subsequently exported. This is a key stage in biofilm formation. However, the mechanism by which alginate is able to exit the cell is poorly understood. It is known that alginate crosses the bacterial outer membrane via the membrane porin, AlgE. We have used a combination of crystallographic techniques, molecular dynamics simulations and docking to reveal the molecular details of the export event.

This study is extended by investigating the interactions of AlgE and alginate with the membrane-associated protein AlgK, providing further insight into the workings of the alginate export machinery.

3708-Pos Board B436
Single Microbe Trap and Release using Sub-Microfluidics: Methods and Applications in Biopolymer Trafficking
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We will present our recent results in the microfluidic manipulation of single microorganisms [1], and the enabling applications in intracellular biopolymer trafficking using vesicle photonics principles [2]. Single cell analysis unmasks information inaccessible in population level data, such as cell-to-cell heterogeneity, and intracellular dynamics. Such studies, however, require the isolation and immobilization of individual cells from a culture, which is usually achieved via optical tweezers [3], electro-phoresis [4], and surface immobilization [5]. Higher throughput analysis is possible using microfluidics; however, this is possible primarily for mammalian cells and not the smaller and more motile microbes, such as fungi and bacteria [6]. We addressed this by developing a hybrid fabrication method that enabled microfluidics of sub-micron dimensions. The latter could both trap and release single microbes, such as Escherichia coli bacteria and Yarrowia Lipolytica fungi. The microfluidic operational and performance will be presented experimentally and computationally. Enabling applications within the field of advanced biofuel production will also be presented. Here the focus is the monitoring of intracellular lipid synthesis and trafficking, aiming at understanding the related underlying biophysics.

tissues, which are assumed to secret some factors under the regulation of nerves causing changes in the tissue stiffness. The tissues contain a large amount of extracellular matrix mainly consisting of collagen fibrils, proteoglycans and microfilbrils. The unique properties of these collagenous tissues might be due to lack of permanent associations between the collagen fibrils and the surrounding extracellular matrix. It seems that cross-linking between the fibrils are formed or broken during the change of the stiffness of the tissues. Its molecular mechanisms are, however, not yet fully understood. We isolated a protein factor called 'tensilin' from an extract of sea cucumber body wall dermis, one of the known catch connective tissues. It stiffens the detergent-treated dermal pieces and induces aggregation of collagen fibrils isolated from the tissue. We also isolated another protein factor which stiffens the dermal pieces. It is possible that there are other factors affecting on interactions among dermal fibrils and the stiffness of the tissues. Molecular mechanisms of the stiffness changes of the catch connective tissues should be clarified by purifying and characterizing these factors.

**3711-Pos Board B439**

**Diffusion Discrepancy between Stroma of Tumor and Normal Tissues**

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It is known that stromal microenvironments change in terms of its structure and composition during tumor progresses. Such change can lead to changes of diffusion efficiency and/or orientation of small molecules. Given small molecules such as cytokines and microRNAs are actively involved in tumor-igenesis, study diffusion in tumor stroma can lead to identifying the mechanism contributing to tumor progression. We used fluorescence recovery after photobleaching (FRAP) to examine the immobile fraction, diffusion rates, diffusion directionalities of dextran between 10 kD and 100 kD of molecular weight in stroma from both normal and tumor tissues from human breasts. We found that in the area with dense fibers, the diffusion rate in the tumor tissue is at least 2 fold-higher compared to the normal tissue. Furthermore, it was observed that 20% more dextran is immobilized in the tumor tissue, compared to the normal tissue, during the time frame of FRAP experiment, indicating the existence of efficient physical traps of small molecules in tumor stroma.

**3712-Pos Board B440**

**Effect of Oligosaccharide Modified Material X on Viability of Human Cancer Cell Lines**

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Anticarcinoma agents take part in the selective destruction of cancer cell lines, or inhibit the growth and proliferation of cancer cells. Finding anticarcinoma agents that have not have noteworthy negative side effects is important matter for application in various fields. Most of polysaccharides were used as medical product or an additive to health functional food. For example chitin and chitosan are known to inhibit the growth and proliferation of cancer cells. Recently, we isolated a protein factor called 'tensilin' from an extract of sea cucumber body wall dermis, one of the known catch connective tissues. It stiffens the detergent-treated dermal pieces and induces aggregation of collagen fibrils isolated from the tissue. We also isolated another protein factor which stiffens the dermal pieces. It is possible that there are other factors affecting on interactions among dermal fibrils and the stiffness of the tissues. Molecular mechanisms of the stiffness changes of the catch connective tissues should be clarified by purifying and characterizing these factors.

**3714-Pos Board B442**

**In Vivo Studies of Active Processes in the Escherichia Coli Nucleoid**

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The cell is the site of actively motor-driven processes which drive the intracellular environment far from thermodynamic equilibrium. The dynamics of biological macromolecules such as DNA in such an environment are complex and subject to a multitude of constraints and forces. Inspired by our in vitro studies of DNA loops with optical tweezers that showed that additional non-thermal fluctuations in the DNA can substantially enhance the formation of regulatory DNA-protein complexes, we study the conformational fluctuations of chromosomal DNA in vivo in Escherichia coli by Fluorescence Correlation Spectroscopy (FCS).

Conformational fluctuations of the DNA move the fluorophores stochastically into the diffusion-limited excitation volume of a focused laser beam in a confocal microscope. From the time correlation functions of the measured fluorescence intensity, we quantify the fluctuations of the DNA as measured by its time-dependent mean square displacement, and the viscous-elastic moduli of the nucleoid. These quantities in live cells significantly differ from the ATP-depleted dead cells on longer time scales, indicating that the fluctuations on longer time scale may be driven by active processes involving molecular motors that generate forces by ATP hydrolysis. On shorter time scales, we see little difference between live and dead cells, suggesting that these processes on corresponding short length scales rely primarily on thermally-driven diffusive mechanisms. We also note that the rheological properties of E. coli nucleoid significantly change when the ATP hydrolysis in cells is inhibited.

**3715-Pos Board B443**

**The Energetic Contribution of Water in the Binding of Ribonuclease A and UMP**

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In most treatments of aqueous binding reactions, the energetic contribution of water is not addressed explicitly by the governing equation. The classical equation for a binding equilibrium \( \Delta G^\circ = -RT\ln K \) may be appropriate at infinite dilution but not under experimental conditions, especially in "nonideal" solutions containing other solutes. Resolving this issue is paramount to understanding the thermodynamics of molecular interactions in the context of a living cell. In the current study, we test a new equation that treats water as a co-reactant and co-product of the balanced reaction. The binding affinity of ribonuclease A (RNase A) with an inhibitor molecule, uridine-3'-monophosphate (UMP), is quantified using isothermal titration calorimetry. The results indicate that the equilibrium "constant," \( K \), is dependent on reactant concentration and that the desolvation energy of binding is unfavorable for this specific protein-ligand interaction. These observations are consistent with published findings for another model binding system, the chelation of calcium by EDTA \( \text{J. Phys. Chem. B} \ 2013, \ 117, \ 8180 \).

**3716-Pos Board B444**

**Size, Stoichiometry, and Organization of Soluble LC3-Associated Complexes**

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