

Biopolymers in Vivo

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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-high 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 uropathogenic *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.

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A Conformational Landscape for Alginate Secretion Across the Outer Membrane of *Pseudomonas aeruginosa*

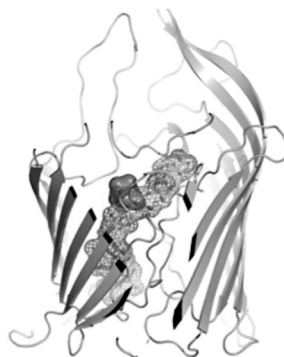
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The exopolysaccharide alginate is a key component of the biofilms produced by *Pseudomonas aeruginosa*, a major pathogen that contributes to the demise of cystic fibrosis patients. Biofilm formation is a major factor in determining the stability and persistence of these bacteria.

Alginate is synthesised 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.



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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 microbes [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 usually is achieved via optical tweezers [3], electrophoresis [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.

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A Sea-Cucumber Derived Novel Protein that Softens the Cell-Disrupted Catch Connective Tissue through Inhibiting the Interaction between Collagen Fibrils

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The dermis in the holothurian body wall is a typical catch connective tissue that shows rapid stiffness changes. The molecular mechanisms of the changes are yet to be clarified. Detection of chemical factors that change stiffness by working directly on the extracellular matrix is vital to clarify the molecular mechanisms. We isolated from the body wall of the sea cucumber *Stichopus chloronotus* a novel glycoprotein, softenin, that softened the body-wall dermis whose cells had been disrupted by Triton X-100. This implied that softenin changed dermal stiffness through directly affecting stiffness of extracellular materials. The apparent molecular mass of softenin was 20 kDa. The N-terminal sequence of 17 amino acids had low homology to that of known proteins. Catch connective tissue takes three different mechanical states, soft, standard, and stiff. Triton-treated dermis corresponded to the dermis in the standard state. Triton-Freeze-Thaw (TFT) dermis, which was prepared by repetitive freeze-and-thawing of Triton-treated dermis, corresponded to the soft dermis. TFT dermis did not respond to softenin whereas it was stiffened by tensilin, a sea-cucumber derived stiffening protein. Tensilin-stiffened TFT dermis became soft when softenin was applied, suggesting the antagonism between softenin and tensilin. The antagonism was also observed in the suspended solution of collagen fibrils isolated from the sea-cucumber dermis. Tensilin caused fibril aggregation while softenin suppressed it. Freeze-and-thawing not only caused stiffness change from standard to soft state in Triton-treated dermis but also induced dissolving the tensilin-aggregated collagen fibrils. These results suggested that softenin decreased dermal stiffness through inhibiting some mechanical interactions between collagen fibrils. Thus, softenin is the first softener shown to work directly on the mechanical interaction between extracellular materials.

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Connective Tissues in Echinoderm Animals that can Reversibly Change their Stiffness and their Stiffening Protein Factors

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Some connective tissues shown in echinoderm animals such as sea urchins, sea stars, and sea cucumbers can extensively and reversibly change their stiffness in a few minutes under the regulation of the nervous system. They are called 'catch connective tissues' or 'mutable collagenous tissues'. Neurotransmitters such as acetylcholine, adrenaline, and some oligopeptides cause changes in the stiffness of the living pieces dissected from these tissues. They are no more effective, however, after the treatment with detergent solubilizing cell membranes. There are some granule-containing cells in the