

# Bacterial Mechanics and Mechanobiology

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*Primary CNF Tools Used: ASML, Oxford 100, AJA sputter deposition, VersaLaser, MOS clean anneal*

## Abstract:

Bacteria naturally experience mechanical forces in the environment. Mechanical stresses and strains are generated as bacteria swim in fluids, attach to surfaces, grow in biofilms, and even during normal internal pressure homeostasis. Recent studies have shown that bacteria can sense and respond to mechanical forces, and mechanical stress and strain can influence cell division, cell shape, virulence, biofilm initiation, motility, and toxin resistance. However, because of the small scale of bacteria, it is a challenge to apply controlled mechanical stimuli on a single cell level. We developed a microfluidic platform to apply mechanical loads to single bacteria cells *in vivo*. We used this microfluidic platform as a method of applying mechanical stimuli to *Escherichia coli* (*E. coli*) and determining how mechanical stress affects a group bacterial cell envelope proteins used for toxin and antibiotic resistance. In addition, we are using this microfluidic device in the first step toward determining the mechanical properties of the bacterial cell envelope.

## Summary of Research:

Our work involves the use of microfluidic devices as tools for mechanical testing of live bacteria. Within our devices, individual bacteria are flowed into tapered channels and trapped (Figure 1). The bacteria experience mechanical loading from the hydrostatic fluid pressure as well as contact with the tapered channels walls (Figure 2). Key advantages of this microfluidic platform include minimal sample preparation, no chemical immobilization or labeling, the ability to analyze hundreds of cells at once, and the ability to apply different magnitudes of mechanical loading to different bacteria simultaneously [1]. We manufactured devices on silica glass wafers using deep UV photolithography to achieve nanoscale features (250 nm smallest dimension). These glass-on-glass devices were manufactured using the ASML, Oxford 100, AJA sputter deposition, VersaLaser, and MOS clean anneal tools at the Cornell NanoScale Science and Technology Facility.

Recently we have investigated the effects of mechanical stress and strain on the functionality of multicomponent efflux complexes in bacteria. Multicomponent efflux complexes create channels that cross the cell envelope of bacteria and are used to remove toxins including metal ions and antibiotics. Since multicomponent efflux complexes form a rigid link across the cell envelope, we targeted them as being sensitive to changes in cell envelope stress caused by mechanical loading in our microfluidic device. Our data suggests that the assembly and function of the multicomponent efflux

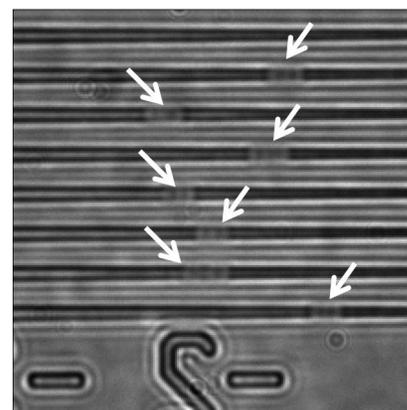


Figure 1: *E. coli* cells trapped within the tapered channels of the microfluidic device. Fluid pressure is used to flow the bacteria into the tapered channels.

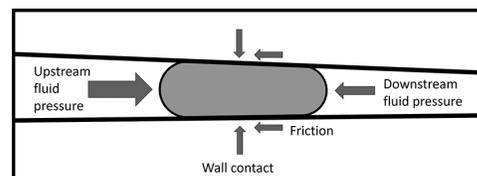


Figure 2: The bacteria cells experiences mechanical loading in the tapered channels due to the hydrostatic pressure, which varies from the upstream end to the downstream end, and due to contact with the walls of the tapered channel.

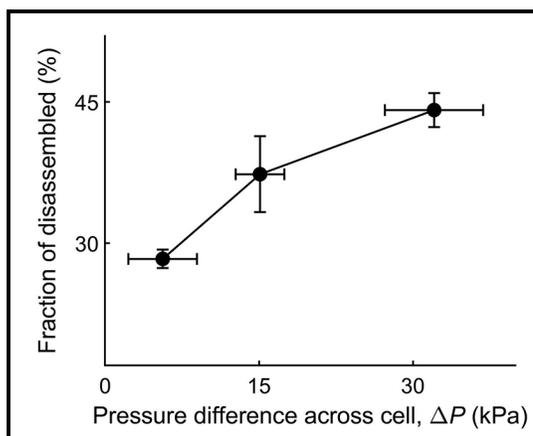


Figure 3: Increased pressure difference across the cell, which correlates with increased applied mechanical load, was shown to increase disassembly of the multicomponent efflux complex CusCBA in *E. coli*.

complex CusCBA in *E. coli* is impaired by increased mechanical stress. Increased applied mechanical stress due to increased pressure in our microfluidic device was shown to promote disassembly of the CusCBA efflux complex (Figure 3). Disassembled CusCBA complexes are nonfunctional and incapable exporting copper ion toxins, suggesting copper ion resistance of mechanically stressed cells is reduced [2]. We are currently investigating if mechanical stress in the cell envelope affects other trans-envelope multicomponent complexes. Preliminary evidence shows that assembly of the multicomponent efflux complex MacABTolC, a multicomponent efflux complex that contributes to macrolide antibiotic resistance, is also sensitive to mechanical stress [3].

### Conclusions and Future Steps:

So far, we have seen that in *E. coli* mechanical stress and strain impairs the proper assembly and function of some of the cellular machinery needed for toxin and antibiotic resistance. In the future we will focus on using our microfluidic device to quantify the mechanical properties of the bacterial cell envelope. Establishing a reliable method of measuring the mechanical properties of the bacterial cell envelope will help us identify subcellular components that contribute to bacterial mechanics as well as how different environmental factors such as antibiotic treatment can change bacterial

mechanical properties. Measuring bacterial mechanical properties has historically been quite challenging and has mostly been limited to atomic force microscopy measurements using fixed bacteria, which has limitations due to uncertain boundary conditions and difficulties separating internal pressure from membrane elasticity [4]. We are working to better understand bacterial mechanical properties by combining experimental data from the microfluidic devices with finite element modelling to calculate numerical estimates for the Young's Modulus of the bacterial cell envelope.

### References:

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