Nanomechanical Response of Bacteria to Antimicrobials: A Pressing Issue

John R. Dutcher
Department of Physics
University of Guelph

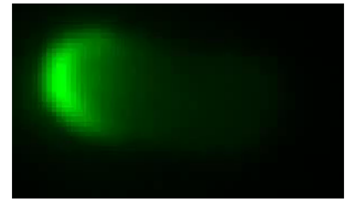
Polymer Surface & Interface Group

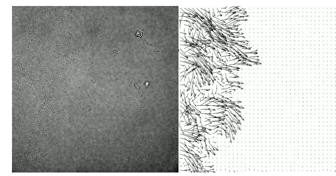
OUTLINE

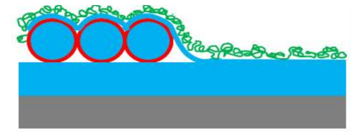
- bacteria basics
  - bacterial cell envelope
- viscoelastic properties of bacterial cells
  - AFM-based creep deformation measurement
    - simple mechanical model
    - comparison of different types of cells
  - effect of cationic antimicrobial compounds
    - “before & after” plus time-resolved measurements
- summary & conclusions

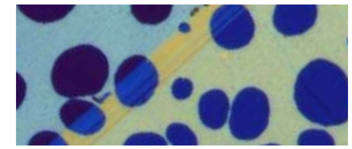
PSI BIOLOGICAL PHYSICS PROJECTS

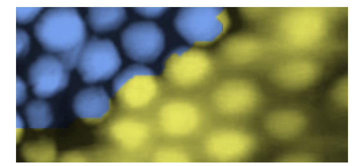
- bacterial biophysics
  - Min protein oscillations & patterns
  - viscoelasticity of bacteria & biofilms
  - twitching motility



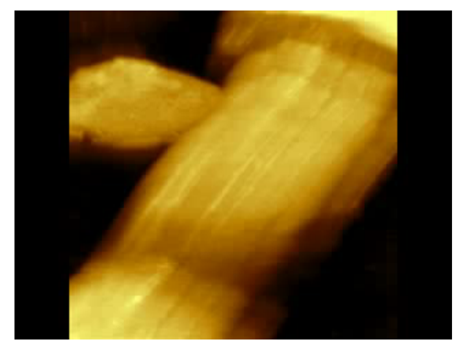








- biopolymers at surfaces & membranes
  - single molecule pulling of proteins on nano-curved surfaces
  - single molecule imaging of peptides in lipids
  - field driven changes in conformation & orientation
- enzymatic degradation of cellulose
  - imaging & kinetics of adsorption & degradation

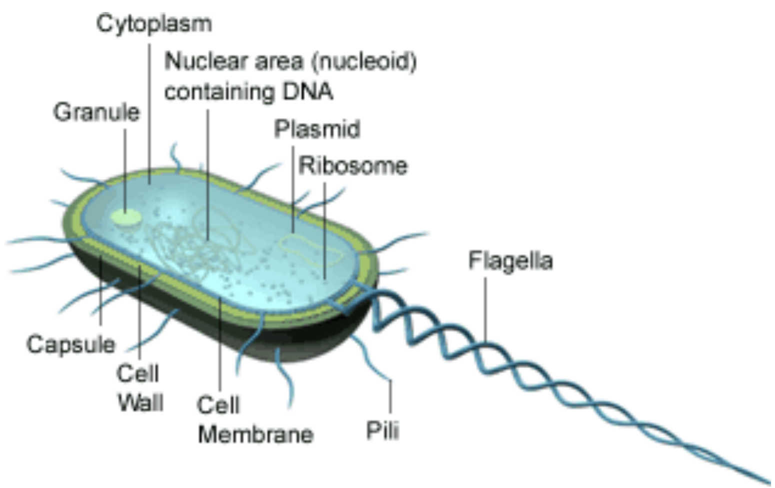


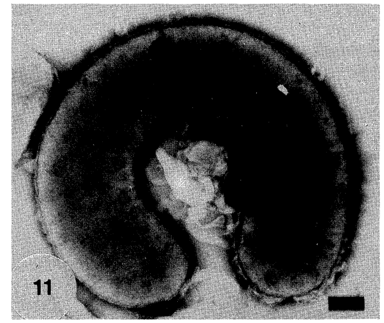
- polysaccharide nanoparticles
  - cool science & startup company

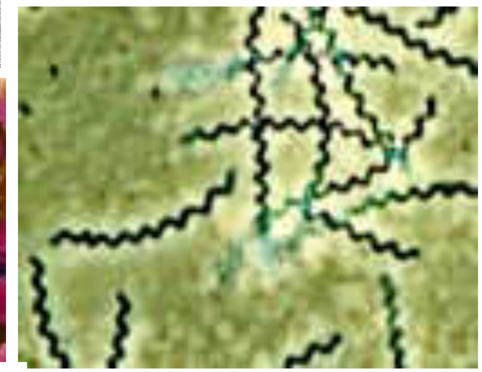
MIREXUS

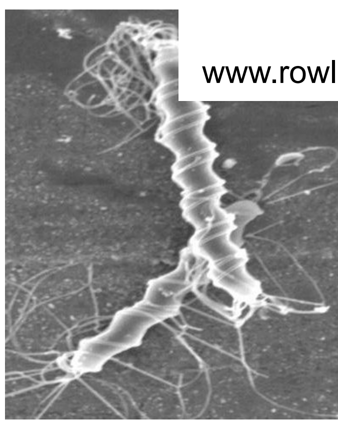
BACTERIA

- many different types & shapes of bacteria in nature



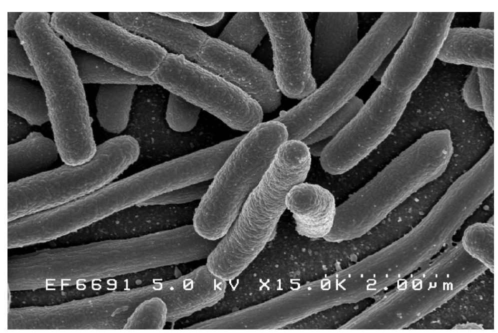


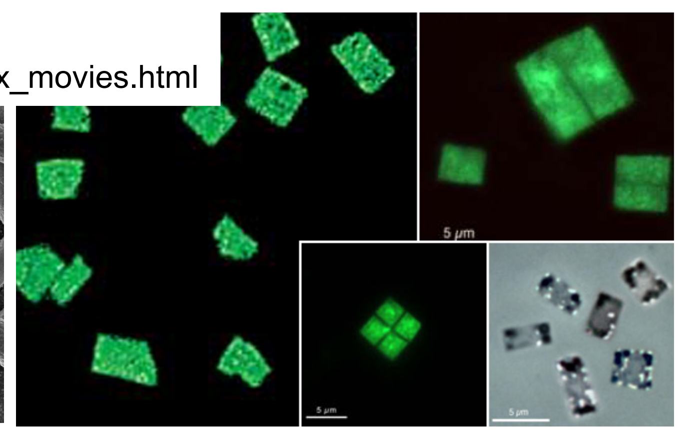




Howard Berg

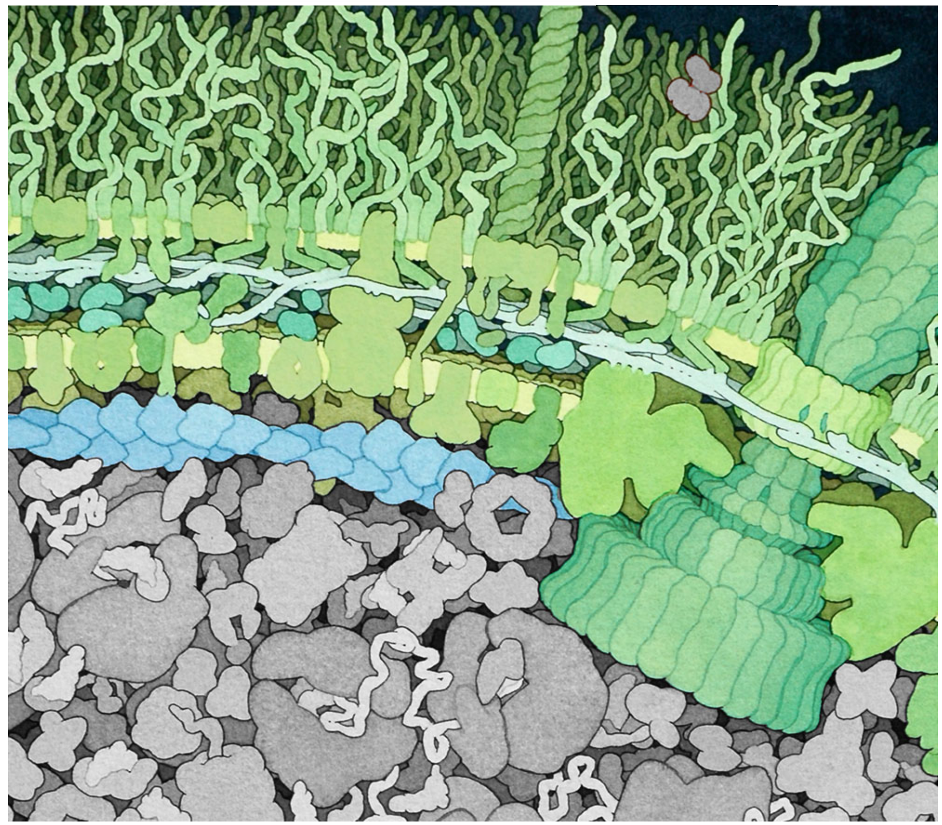
www.rowland.harvard.edu/labs/bacteria/index_movies.html





BACTERIAL CELL ENVELOPE

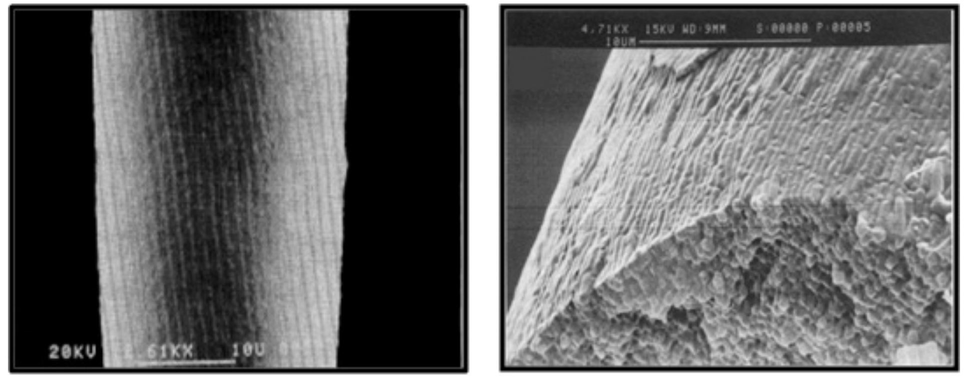
- bacterial cell envelope is boundary with external environment
  - lipid membranes, peptidoglycan, lipoproteins, lipopolysaccharides, etc.
  - cell wall must support turgor pressure, while allowing growth & transport of biomolecules



[David Goodsell, *The Machinery of Life* (2009)]

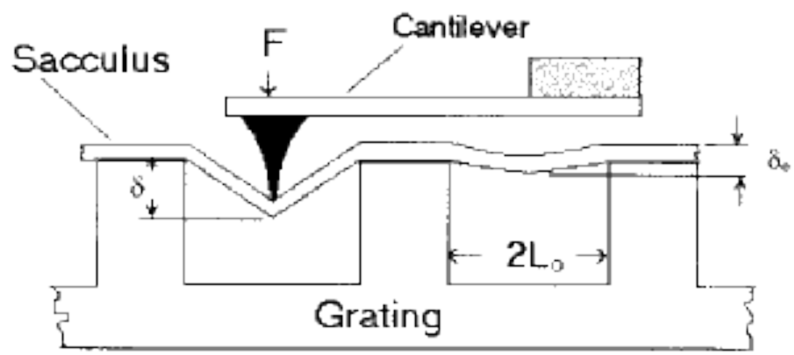
MECHANICAL MEASUREMENTS OF BACTERIA

- first studies of cells
  - changes with pH & ionic strength
  - embedding & stretching in gel strips
  - rupture of cells between flat plate and optical fiber
  - filamentous cells
    → viscoelastic fibers



[Thwaites & Mendelson, PNAS (1985)]

- cell wall components
  - peptidoglycan sacculus
    → elastic modulus



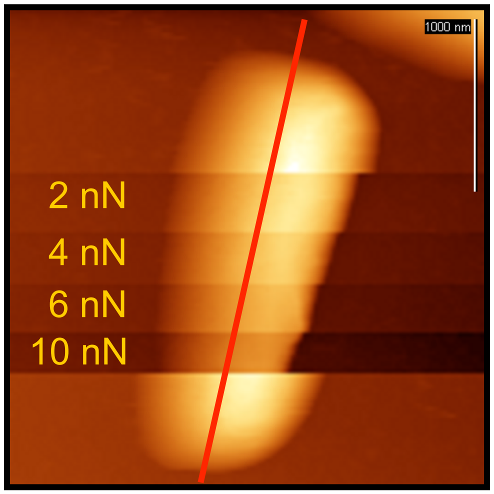
[Yao *et al*., J. Bacteriol. (1999)]

[Vadillo-Rodriguez & Dutcher, Soft Matter (2011)]

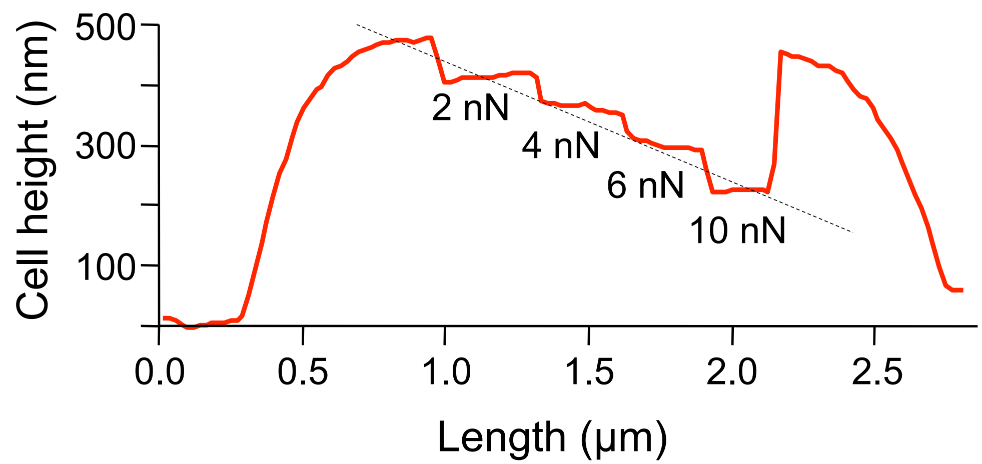
PREPARATION OF BACTERIA FOR AFM

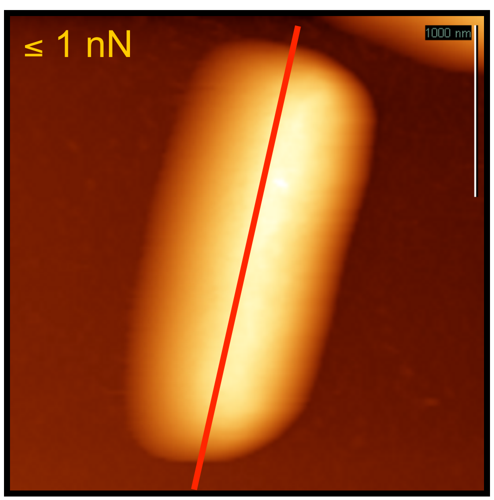
- growth of bacterial cells
  - cultured at 37^{o}C in TSB or LB to late-exponential phase
  - harvested by centrifugation @ 1,150 × g
  - washed twice & re-suspended in deionized water
  - different types of cells
    - Gram negative: *P. aeruginosa* PAO1, *E. coli* (WT, *lpp*)
    - Gram positive: *B. subtilis* 168
- for AFM, bacterial cells must be adhered to a surface
  - use “biological glue”
    - thin, positively-charged polymer layer since cells have negative charge
    - poly-L-lysine, polyethyleneimine, mussel adhesive protein

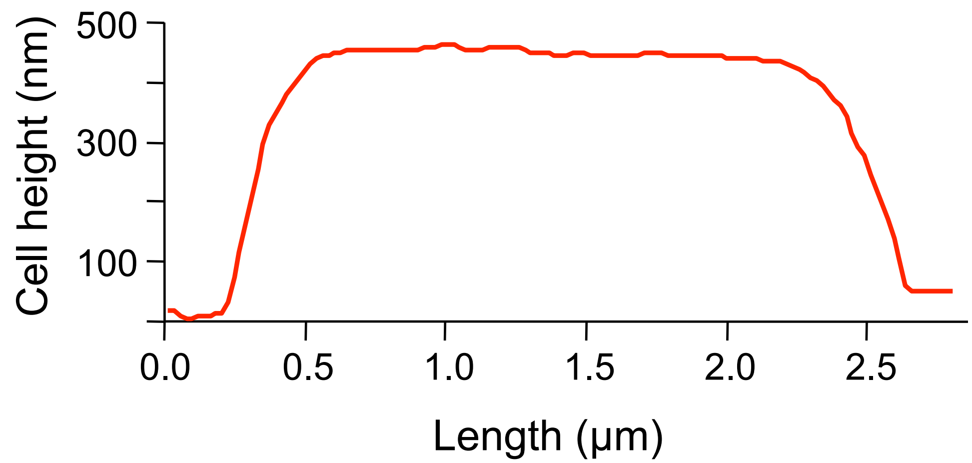
IMAGING AT DIFFERENT FORCES



cells are deformable!



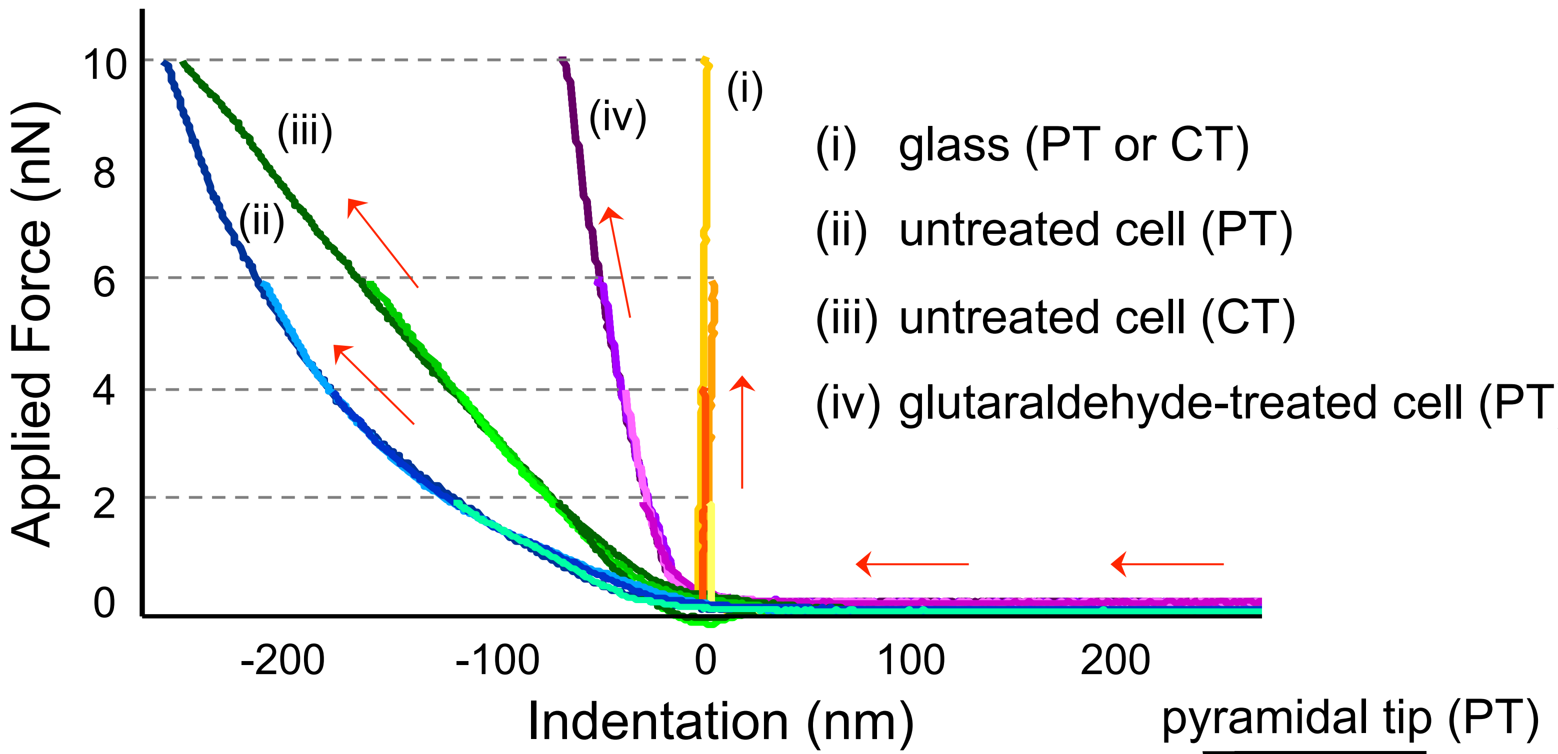


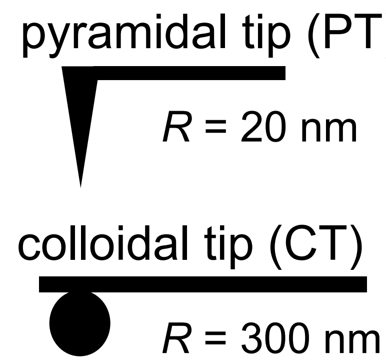


P. aeruginosa PAO1

[Vadillo-Rodriguez *et al.*, J. Bacteriol. (2008)]

FORCE-INDENTATION CURVES





Effective spring constant k_c for linear curves

curve (iii) → $k_c = 0.044 \pm 0.002$ N/m

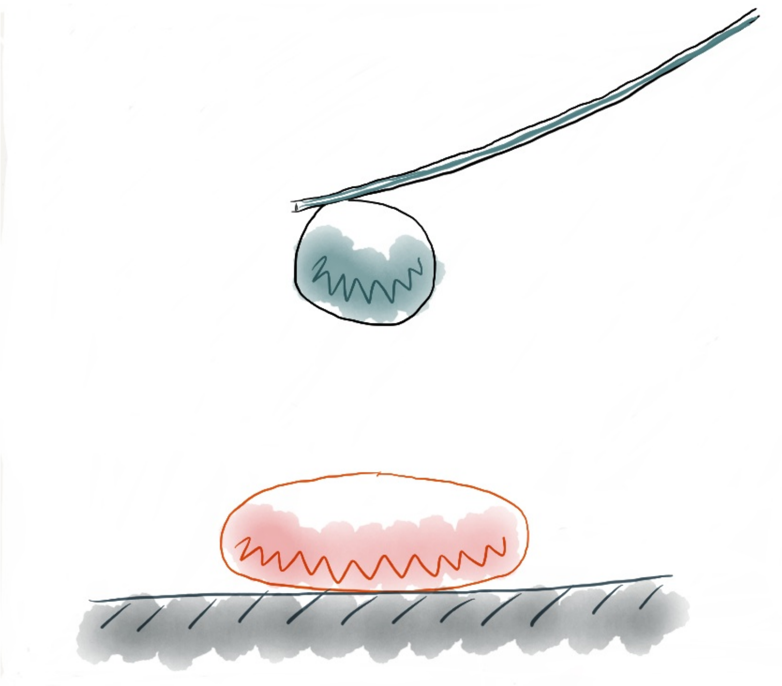
curve (iv) → $k_c = 0.11 \pm 0.03$ N/m

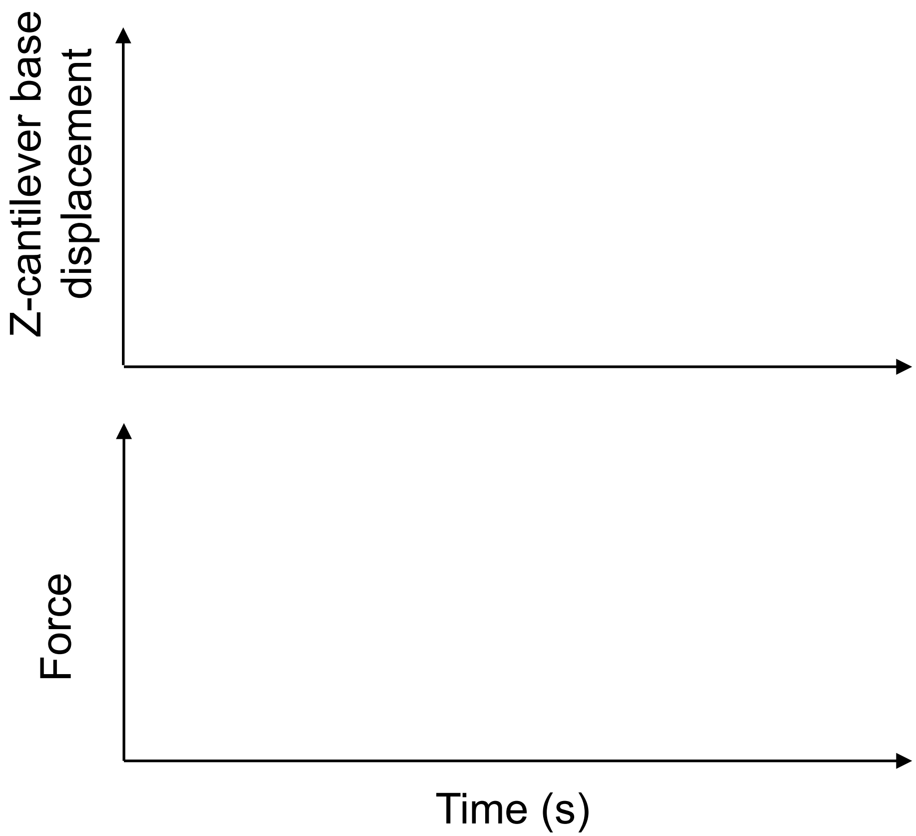
P. aeruginosa PAO1

[Vadillo-Rodriguez *et al*., J. Bacteriol. (2008)]

CREEP DEFORMATION OF BACTERIA

- AFM tip pressing on bacterial cell





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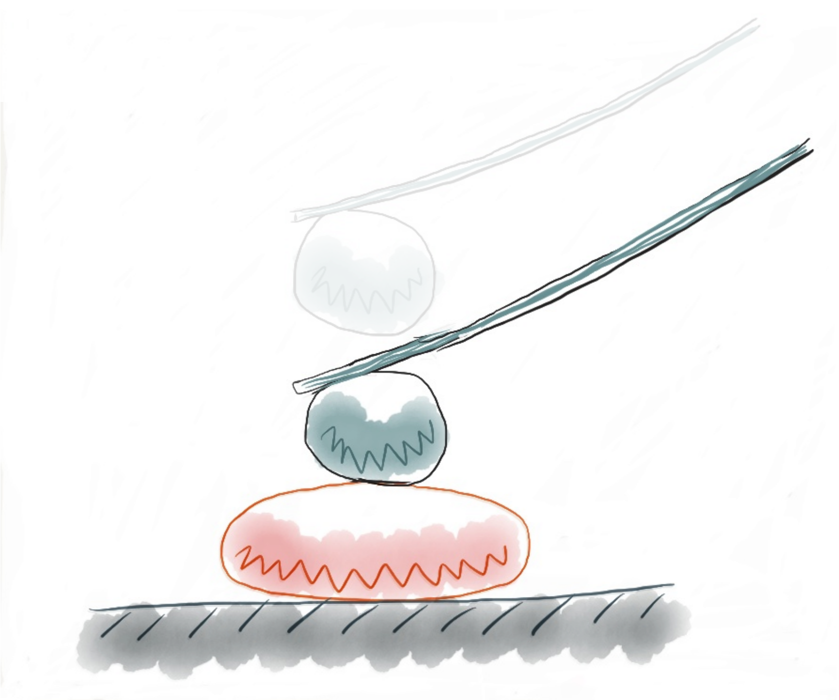

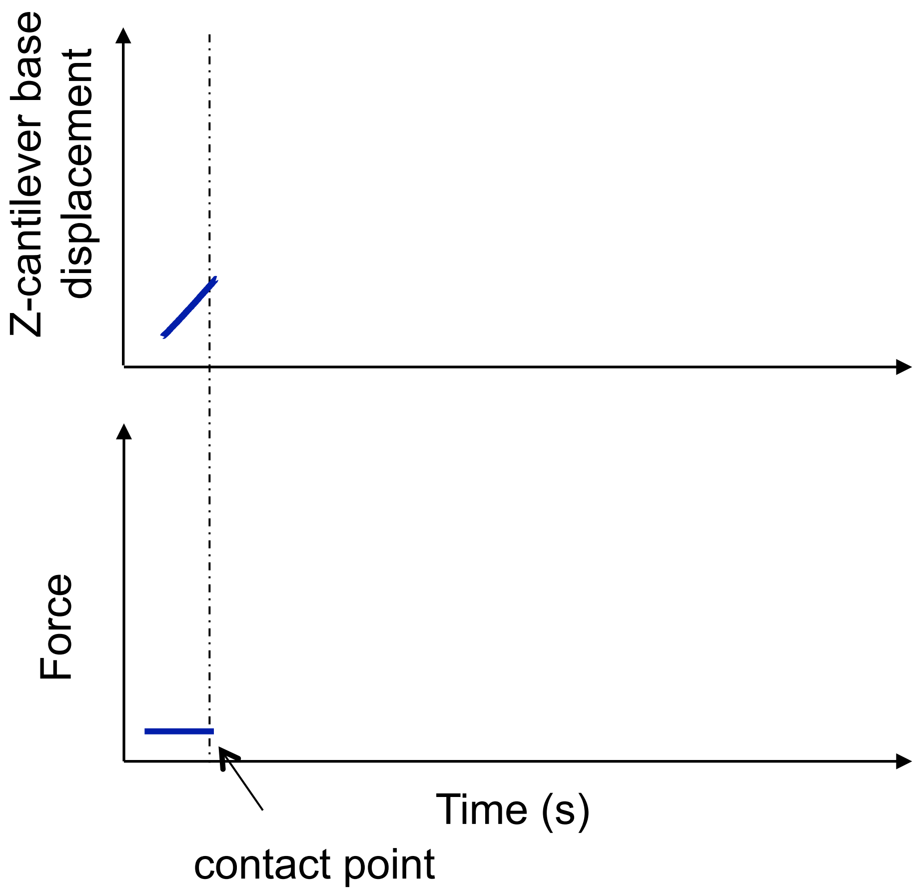

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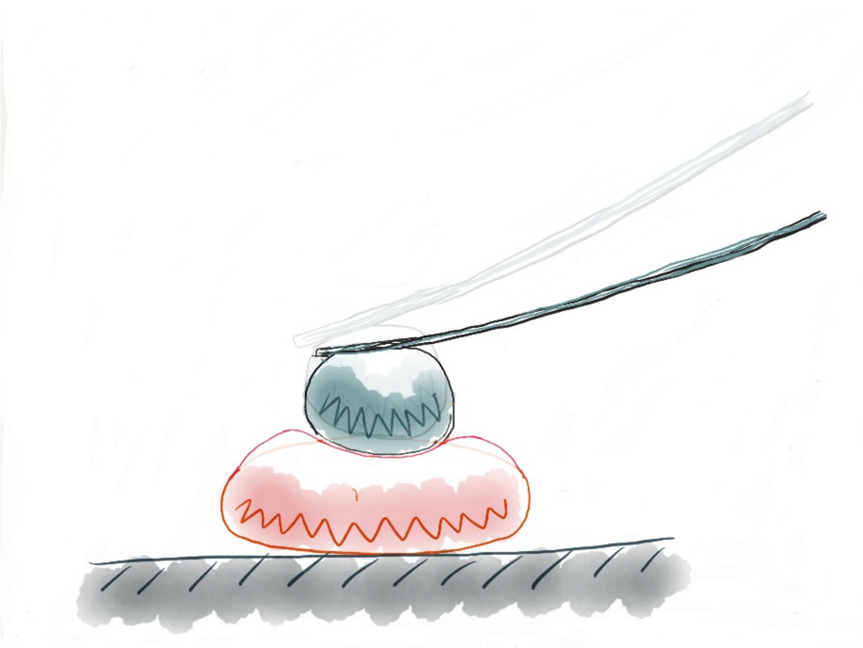

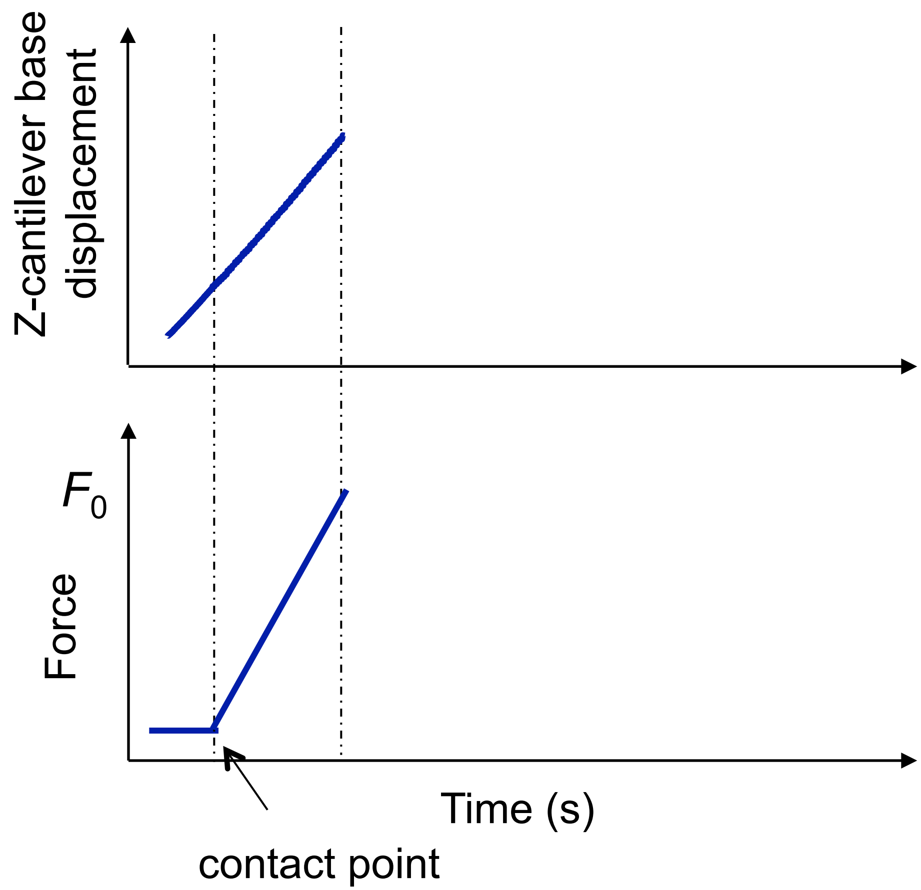

CREEP DEFORMATION OF BACTERIA

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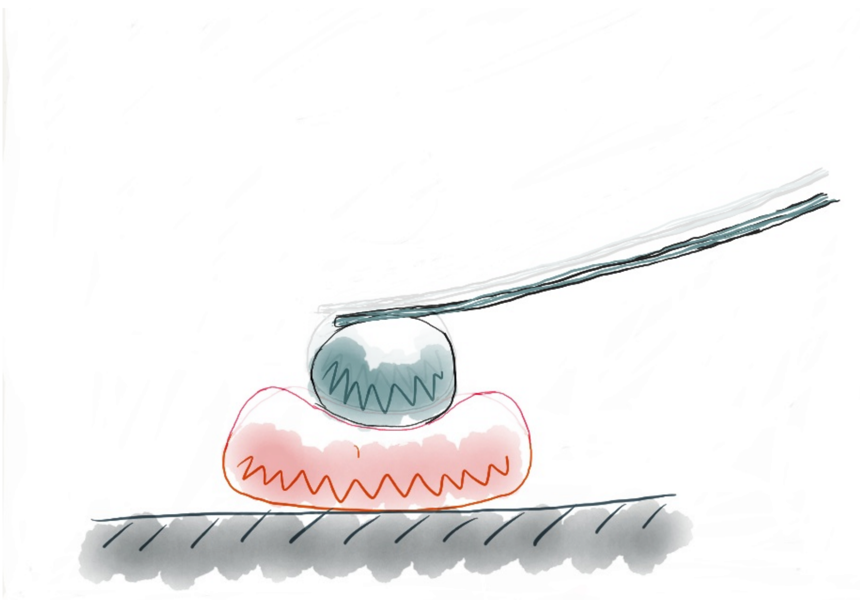
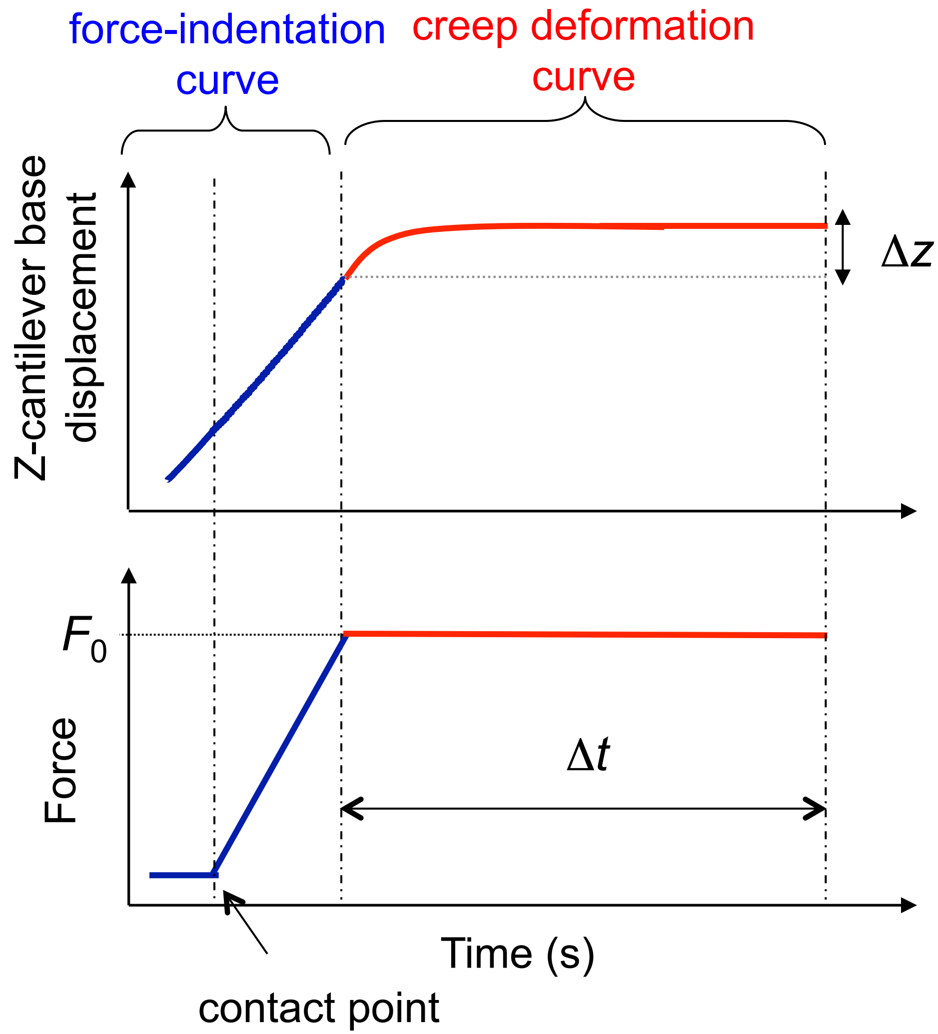
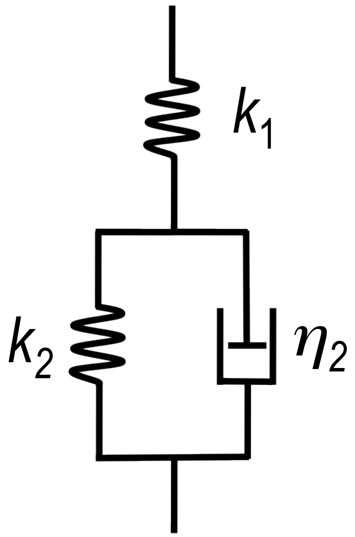




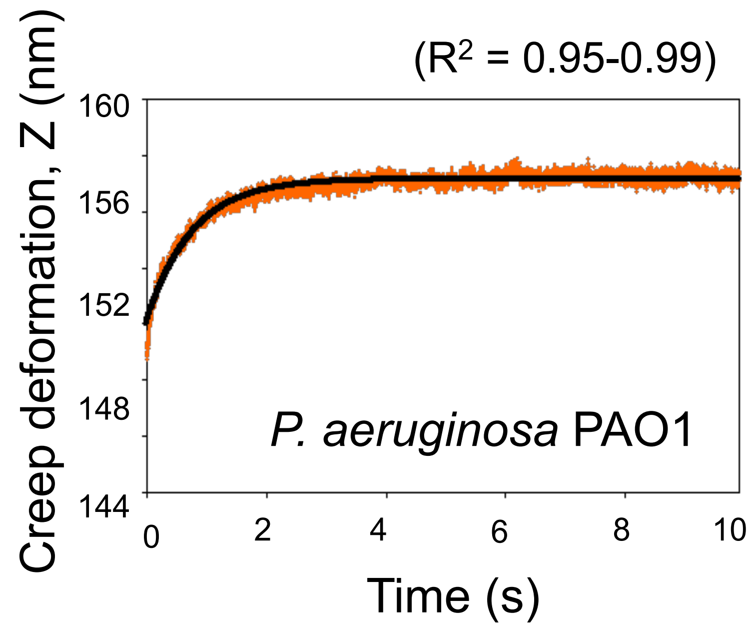
ANALYSIS OF NANOCREEP EXPERIMENT

'Standard solid model'



'Instantaneous' elasticity

'Delayed' elasticity



$$Z(t) = \frac{F_0}{k_1} + \frac{F_0}{k_2}\left[1 - \exp\left(-t\frac{k_2}{\eta_2}\right)\right]$$

$\frac{k_2}{\eta_2} \rightarrow 1/\tau$

Z(t): creep deformation

F_0: applied force

k_1, k_2 : spring constants

η_2 : viscosity

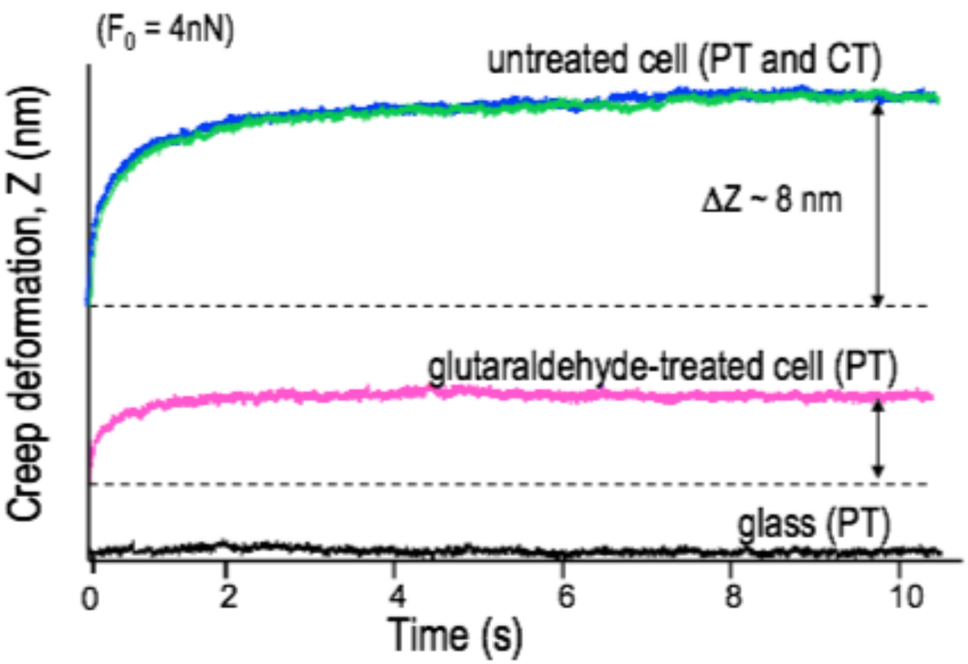
Cell viscoelastic parameters:

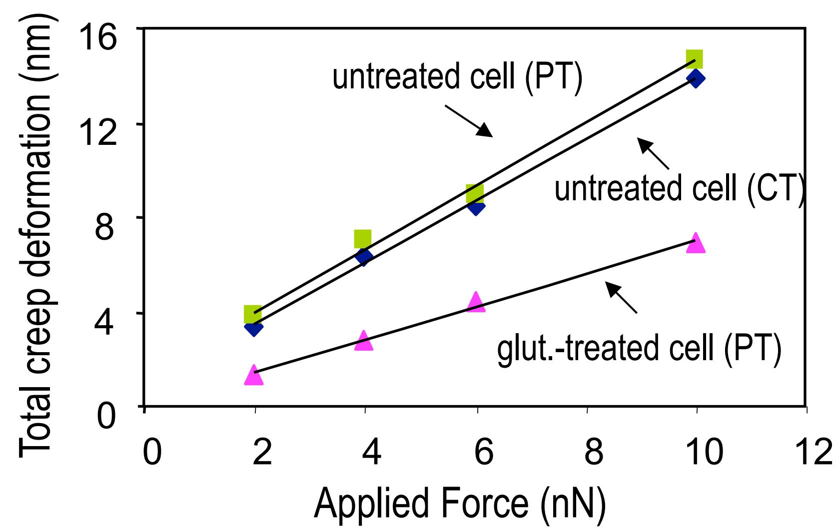
- k_1 : instantaneous elastic constant
- τ : response time

[Vadillo-Rodriguez *et al*., J. Bacteriol. (2008)]

CREEP DEFORMATION CURVES

- check that drift in system is close to zero
- compare results for PT vs CT
- compare untreated cell vs glutaraldehyde-treated cell
  - factor of 2.8 increase in k_1, factor of 2.2 decrease in τ





P. aeruginosa PAO1

[Vadillo-Rodriguez *et al*., J. Bacteriol. (2008)]

CREEP IS A ROBUST PHYSICAL MEASUREMENT

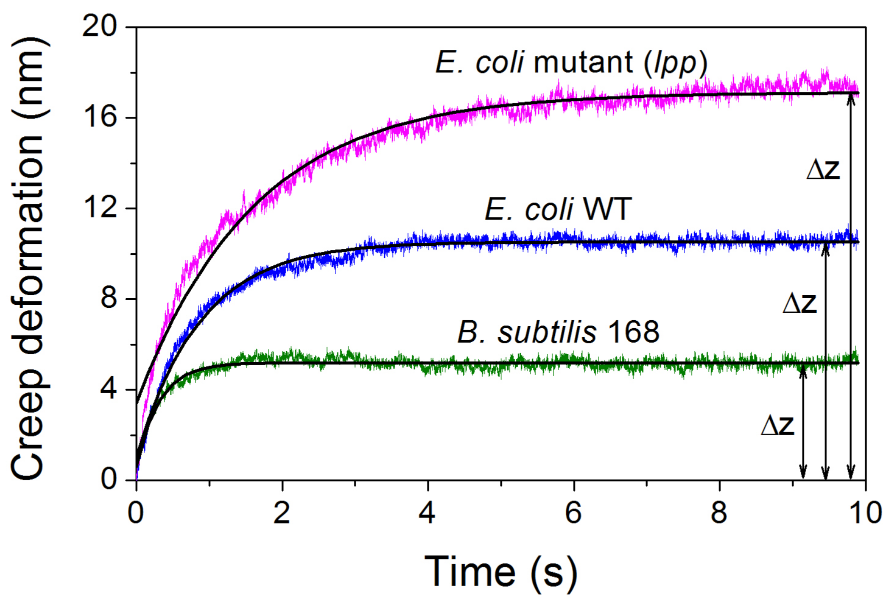
- perform measurement multiple times on many different cells
  - cells measured at same point in life cycle, at centre of cell

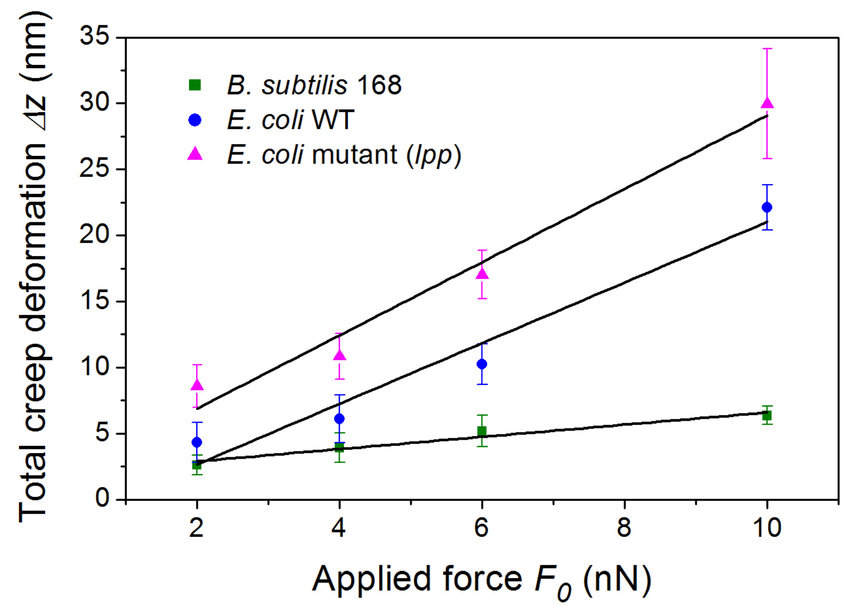
	Untreated cells		Glut.-treated cells
	PT	CT	PT
k_1 (N/m)	0.03 ± 0.01	0.044 ± 0.002	0.11 ± 0.03
τ (s)	1.7 ± 0.2	1.8 ± 0.2	0.8 ± 0.3

⟶ very well-defined and reproducible physical measurement

DIFFERENT TYPES OF CELLS

- compare Gram negative *E. coli* WT with Gram positive *B. subtilis*
  - factor of 2.2 increase in k_1, factor of 1.2 decrease in τ
- compare *E. coli* WT with *E. coli* mutant *lpp* (lipoprotein deficient)
  - factor of 1.7 decrease in k_1, factor of 2.4 increase in τ

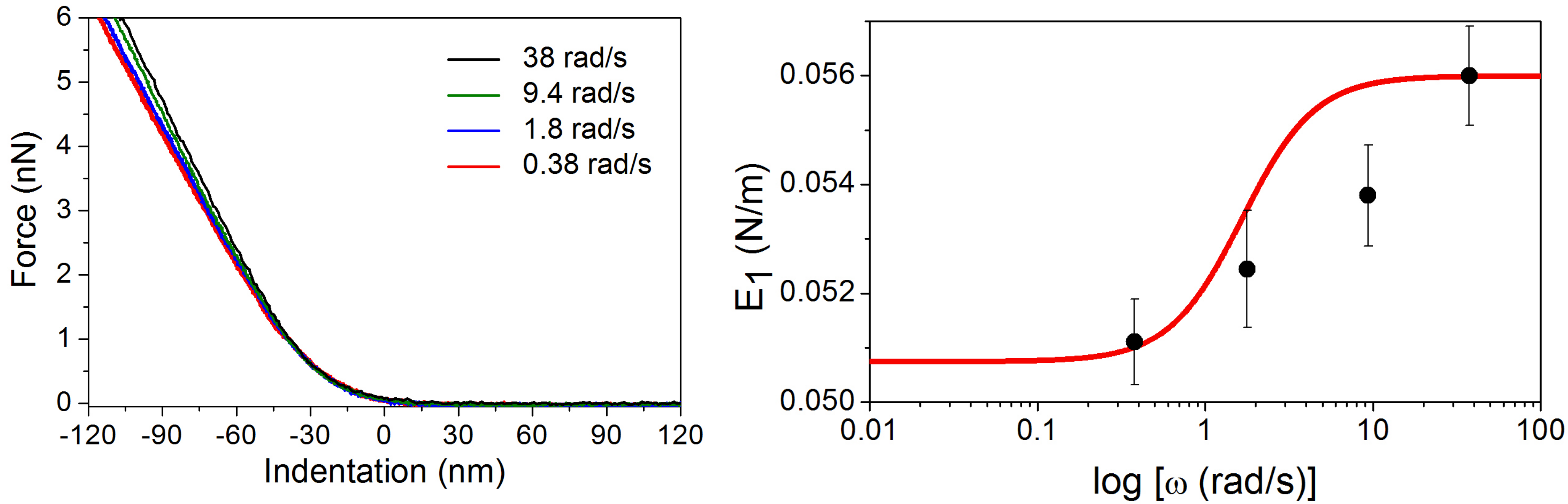




[Vadillo-Rodriguez *et al*., J. Bacteriol. (2009)]

DYNAMIC VISCOELASTICITY

- force-indentation curves
  - vary loading rate for rates comparable to $1/\tau$
- determine elastic modulus E_1 at different loading rates
- compare measured & calculated E_1 values



E. coli K12

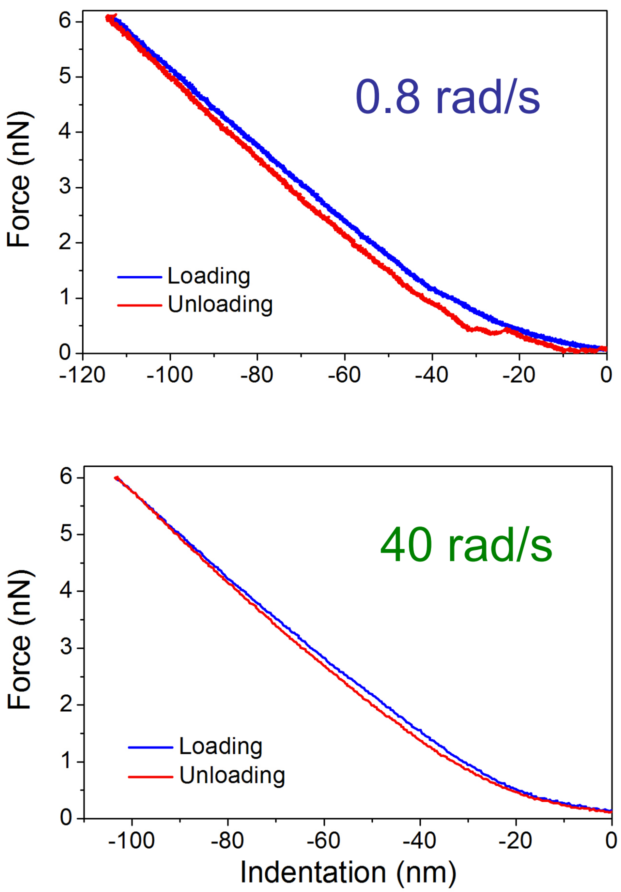
[Vadillo-Rodriguez & Dutcher, Soft Matter (2009)]

DYNAMIC VISCOELASTICITY

– hysteresis in approach & retraction curves

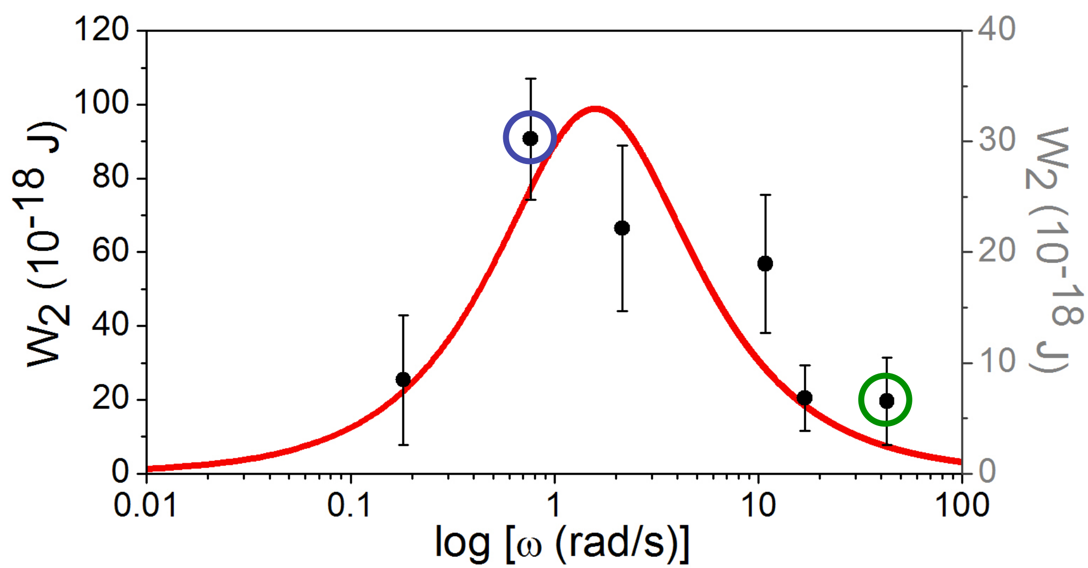
– determine dissipated energy W_2 at different loading rates

– compare measured & calculated W_2 values



E. coli K12

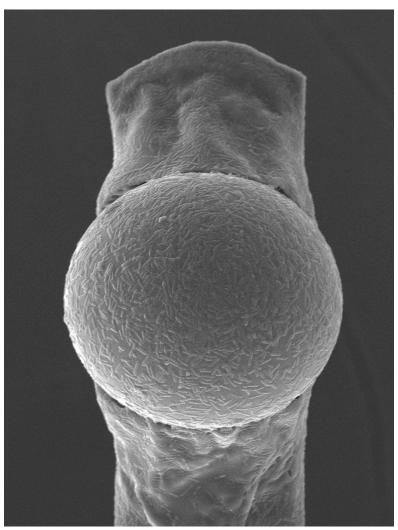
$$W_2 = \pi x_0^2 E_2$$

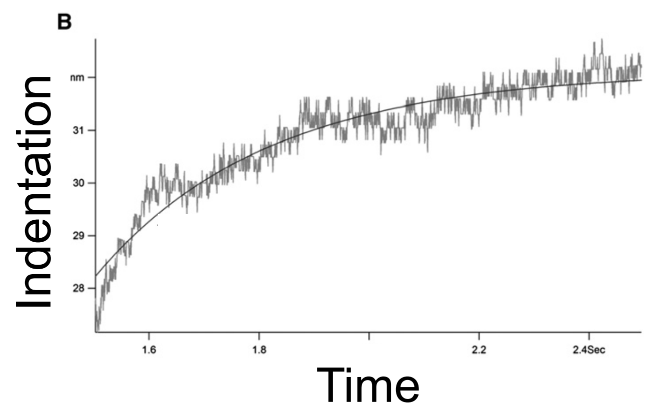


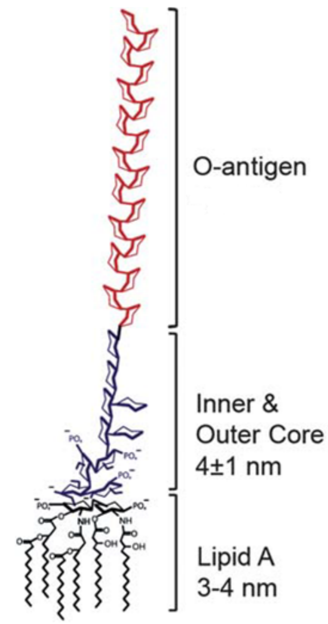
[Vadillo-Rodriguez & Dutcher, Soft Matter (2009)]

BACTERIAL BIOFILMS

- viscoelastic properties of bacterial biofilms
  - compare WT *P. aeruginosa* PAO1 and isogenic LPS mutants
    - coat bead on AFM cantilever with bacterial cells
    - press on clean glass surface & biofilms
      - force-distance curves ➡ stiffness, adhesion, cohesion
      - creep deformation curves ➡ viscoelasticity







[Lau *et al*., Biophys. J. (2009); Lau *et al*., J. Bacteriol. (2009)]

BACTERIAL BIOFILMS

- viscoelastic properties of bacterial biofilms
  - compare WT *P. aeruginosa* PAO1 and isogenic LPS mutants migA, wapR & rmlC, and correlate with confocal microscopy

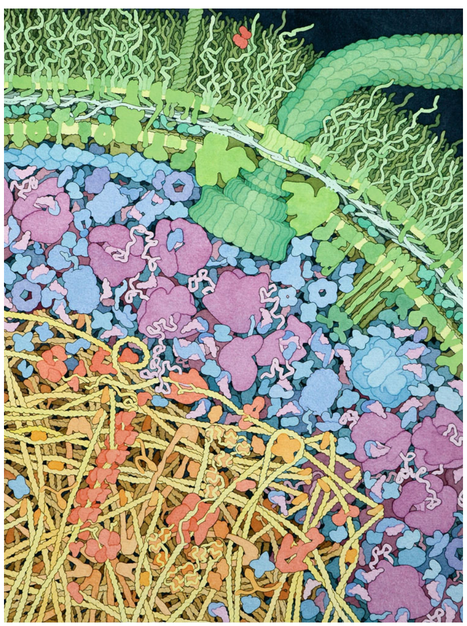
changes in LPS expression → mechanical changes to cells → structural changes to biofilms

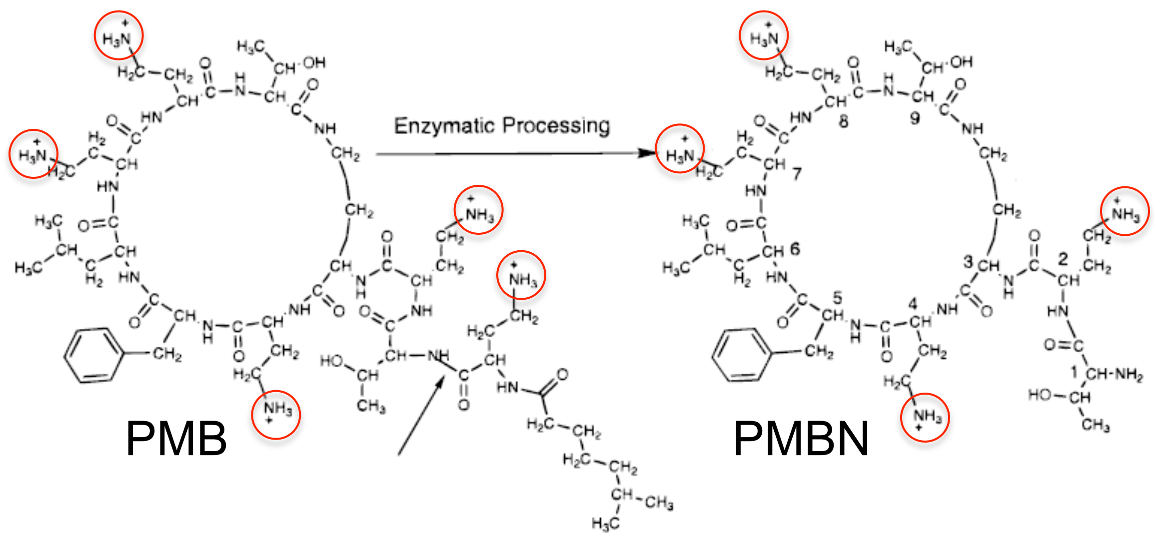
  - differences between early-stage & late-stage biofilms
    - stiffness & adhesion decrease as biofilm ages
  - differences between different mutants
    - wapR biofilms have smaller stiffness & much larger adhesion & cohesion than WT

[Lau *et al*., Biophys. J. (2009); Lau *et al*., J. Bacteriol. (2009)]

ANTIMICROBIAL ACTIVITY

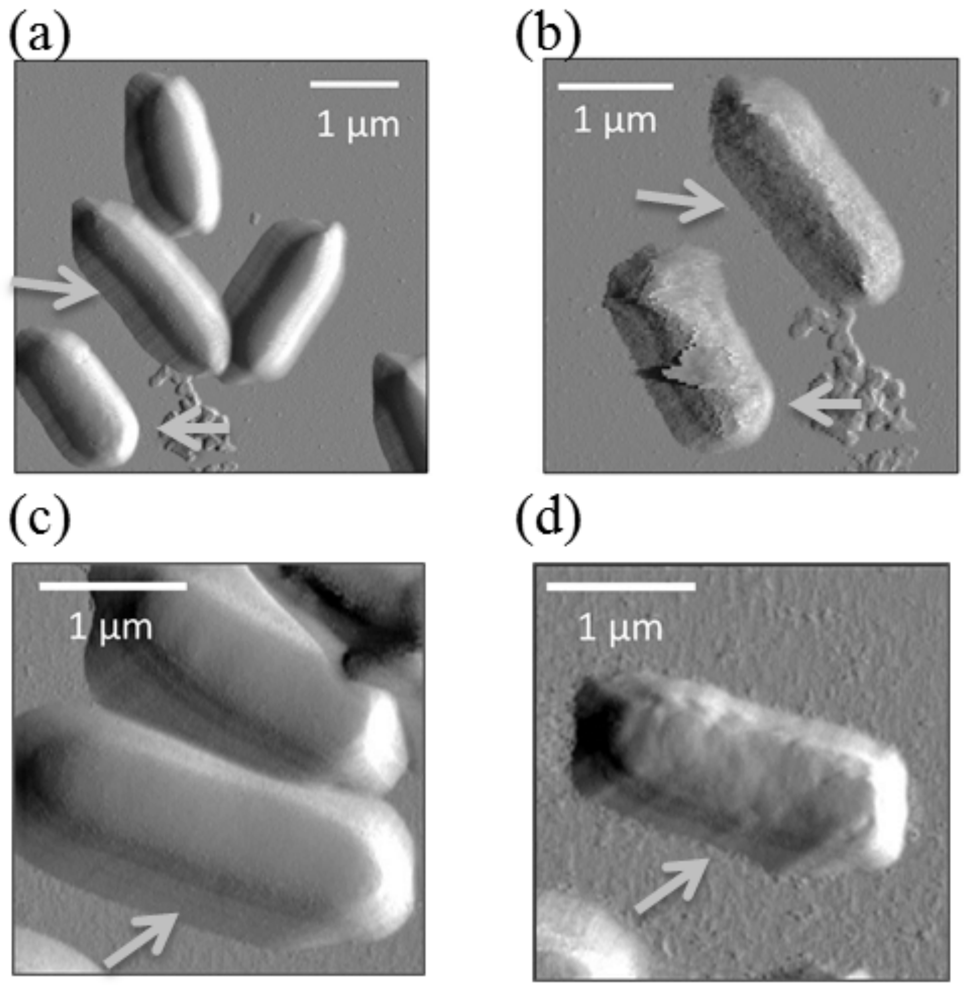
- use creep experiment to evaluate antimicrobial action
  - polymyxin B (PMB) and polymyxin B nonapeptide (PMBN)
    - polymyxins are currently "last hope" antibiotics
    - PMB & PMBN bind to lipopolysaccharide in outer membrane (OM) & change permeability
    - PMB makes it to cytoplasmic membrane





AFM IMAGING OF EFFECT OF PMB & PMBN

before PMB,
1 nm rms
roughness



11 min after
50 µg/mL
PMB,
8 nm rms
roughness

before PMBN,
1 nm rms
roughness

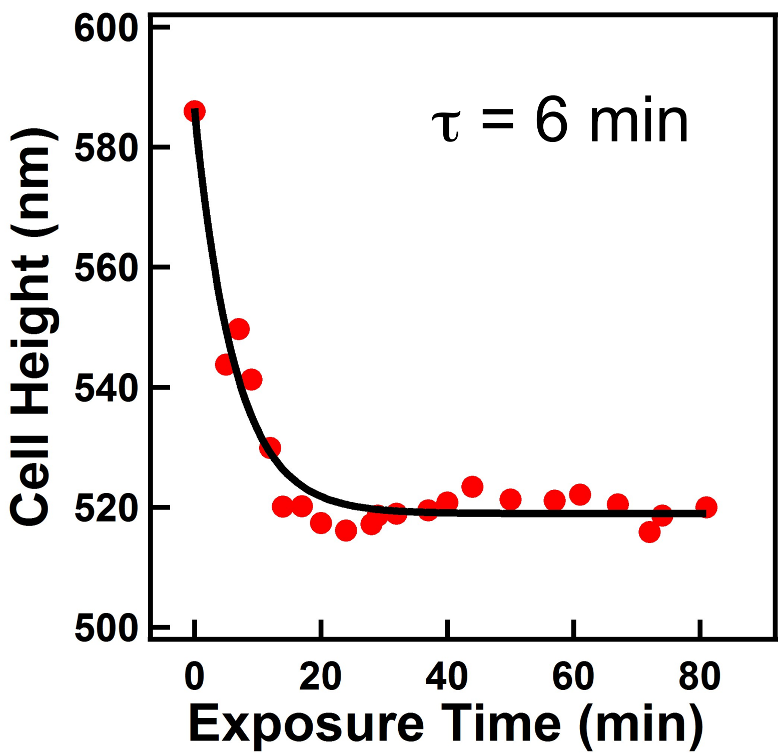
16 min after
50 µg/mL
PMBN,
5 nm rms
roughness

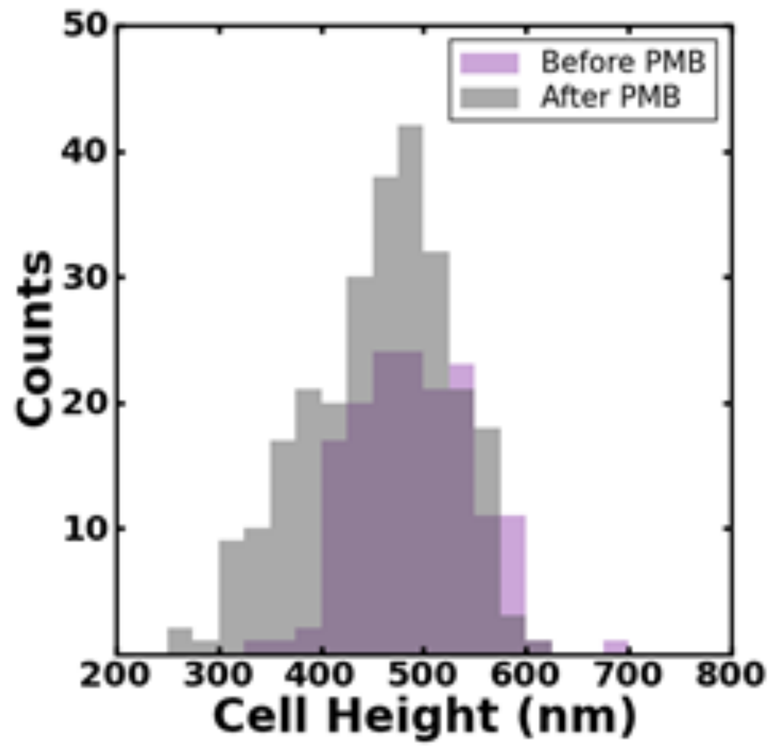
imaged in liquid

[Lu *et al.*, Soft Matter (2014)]

EFFECT OF PMB ON CELL HEIGHT

- small, rapid decrease in cell height





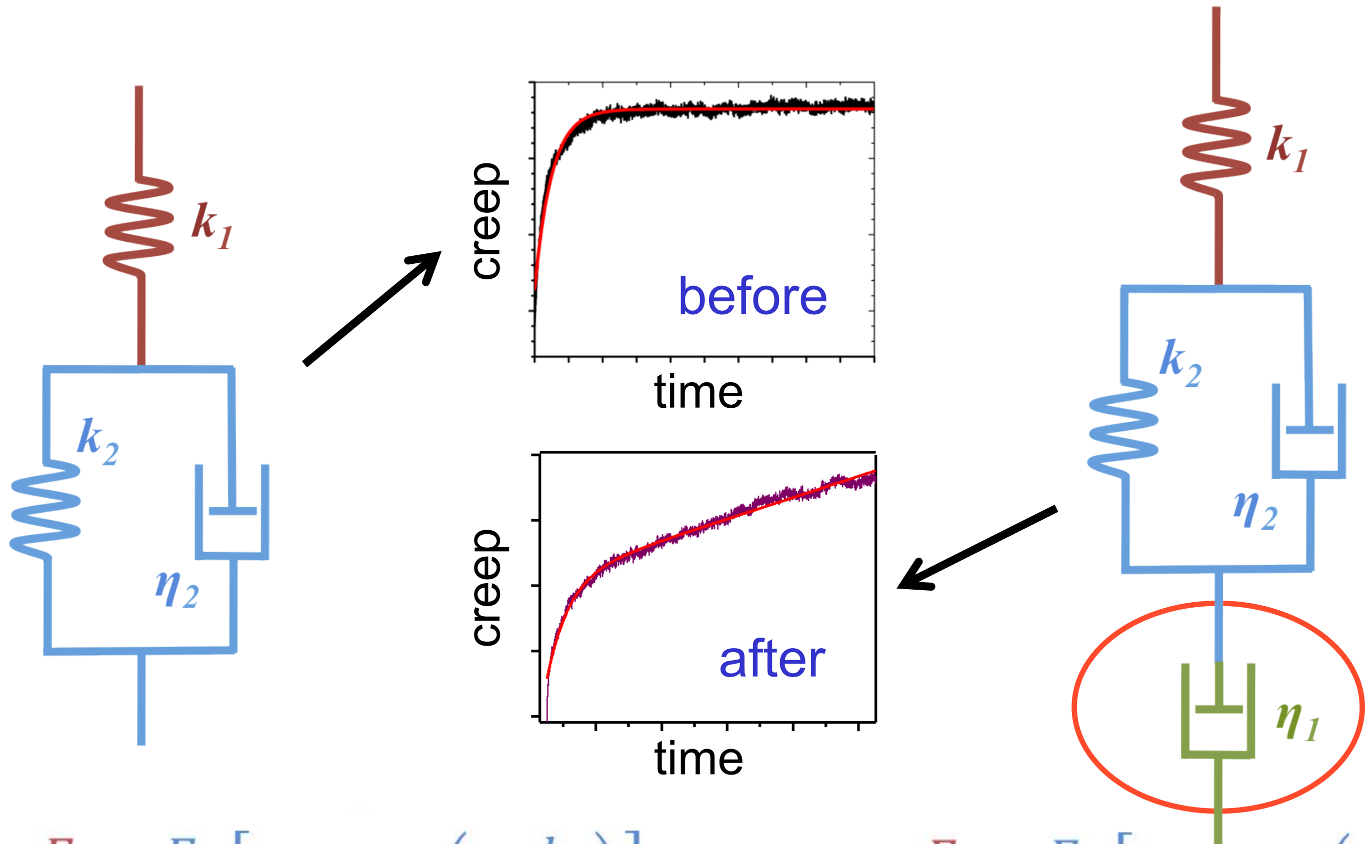
P. aeruginosa PAO1

50 μg/mL PMB

[Lu *et al*., Soft Matter (2014)]

VISCOELASTIC MODELS

- exposure to PMB & PMBN requires use of four element model



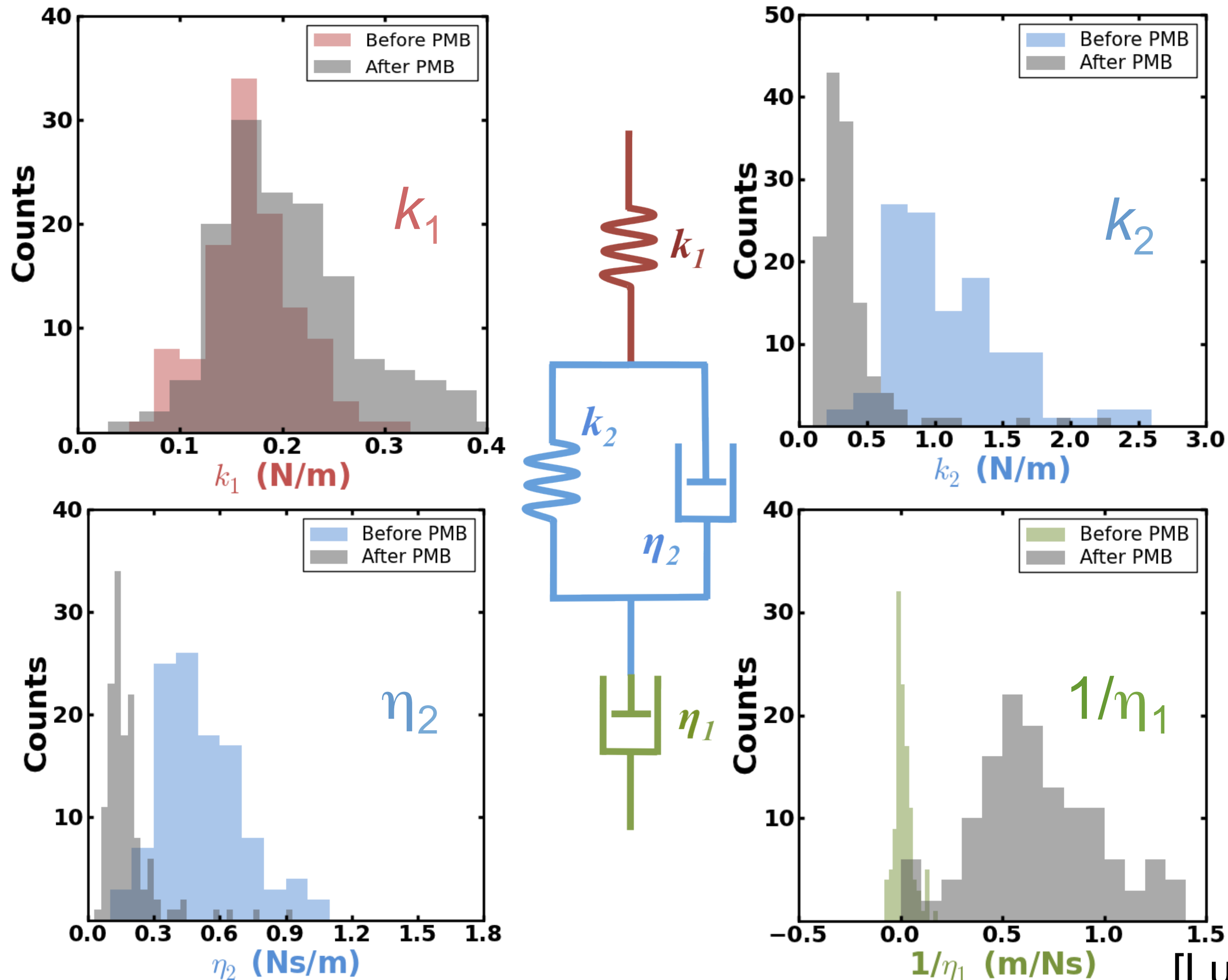
$$Z(t) = \frac{F_0}{k_1} + \frac{F_0}{k_2}\left[1 - \exp\left(-t\frac{k_2}{\eta_2}\right)\right]$$

$$Z(t) = \frac{F_0}{k_1} + \frac{F_0}{k_2}\left[1 - \exp\left(-t\frac{k_2}{\eta_2}\right)\right] + \frac{F_0}{\eta_1}\cdot t$$

[Lu *et al.*, Soft Matter (2014)]

BEFORE & AFTER PMB EXPOSURE

- viscoelastic parameters before & after 1 h exposure to PMB



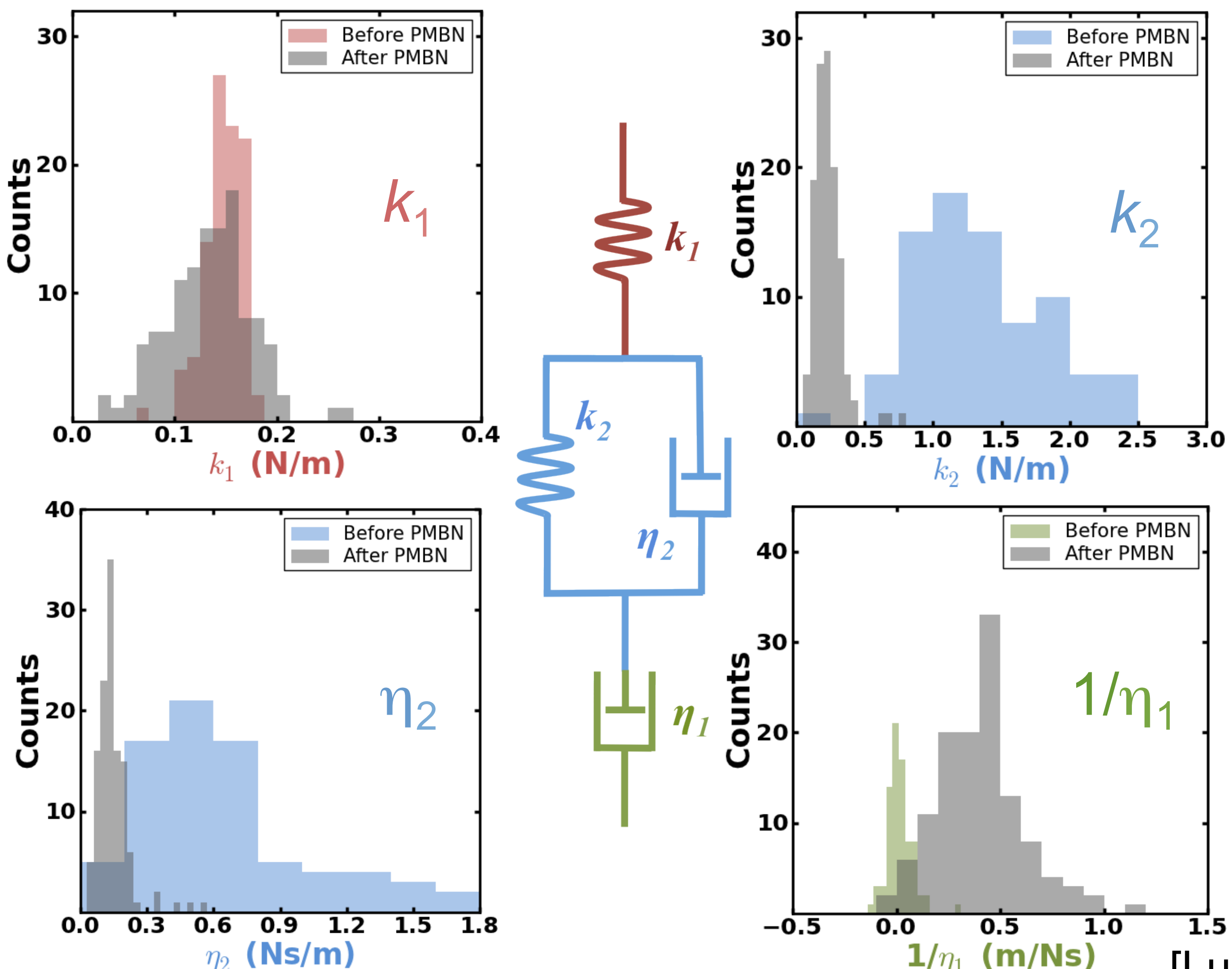
P. aeruginosa PAO1
50 μg/mL PMB

- $1/\eta_1$ provides distinctive signature for loss of integrity
- slight increase in k_1
- large decreases in k_2, η_2

[Lu *et al.*, Soft Matter (2014)]

BEFORE & AFTER PMBN EXPOSURE

- viscoelastic parameters before & after 1 h exposure to PMBN



P. aeruginosa PAO1

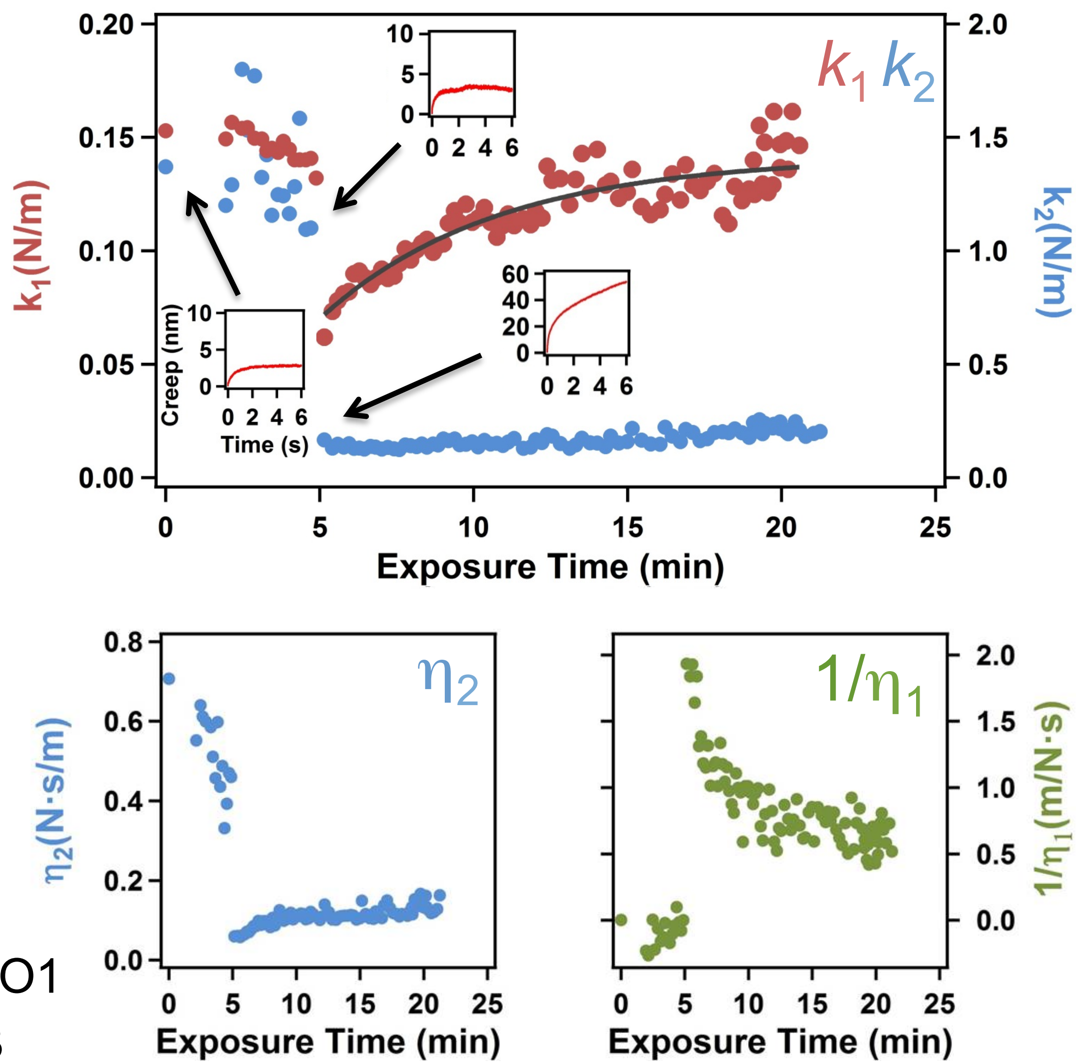
50 μg/mL PMBN

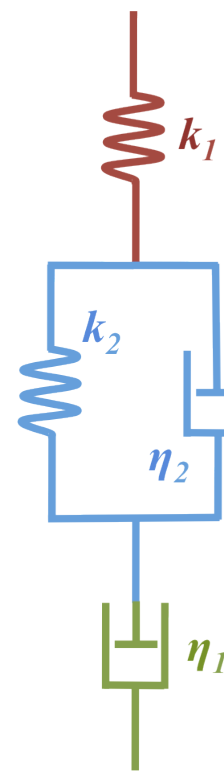
- smaller increase in $1/\eta_1$
- slight decrease in k_1
- large decreases in k_2, η_2

[Lu *et al.*, Soft Matter (2014)]

TIME-RESOLVED RESPONSE TO PMB

- rapid loss of integrity followed by slow recovery



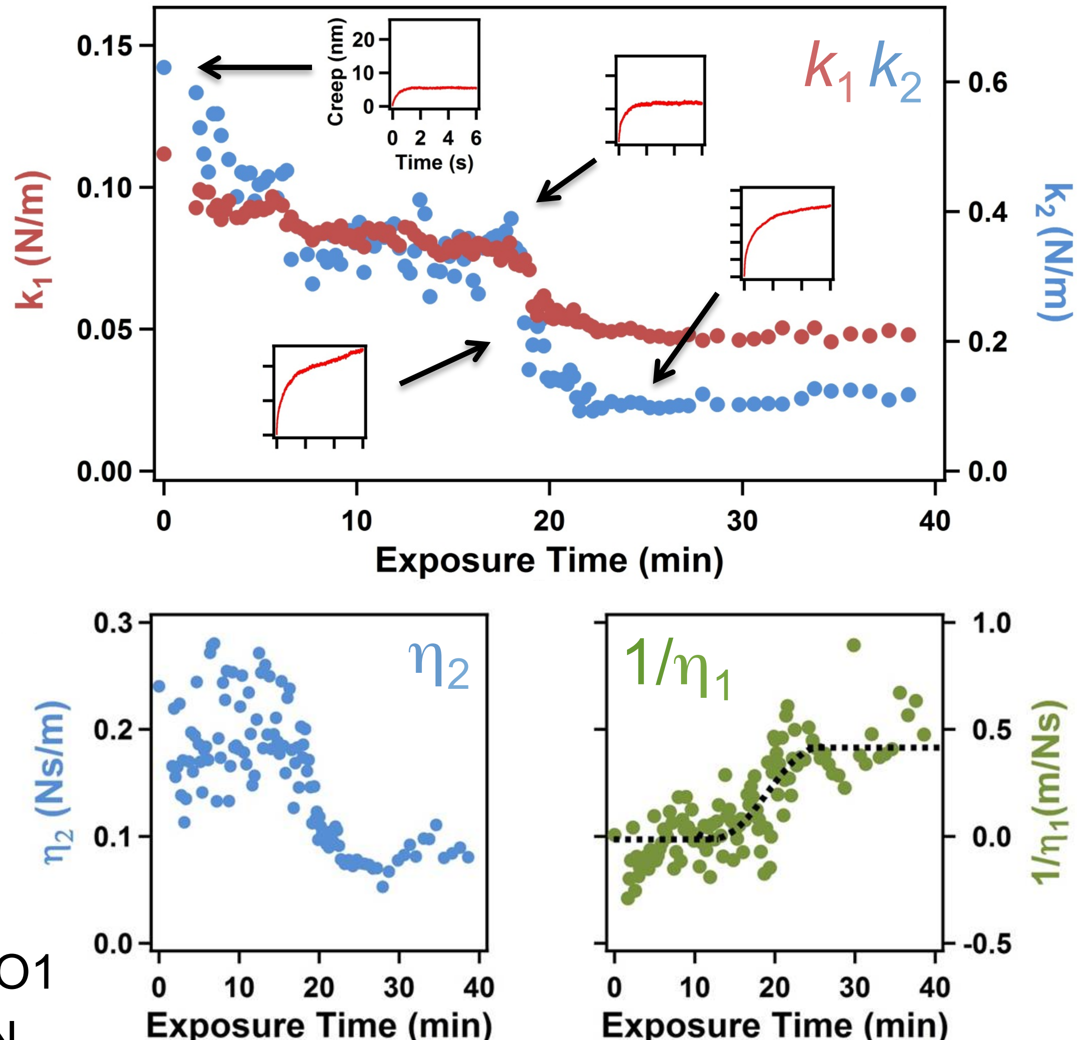


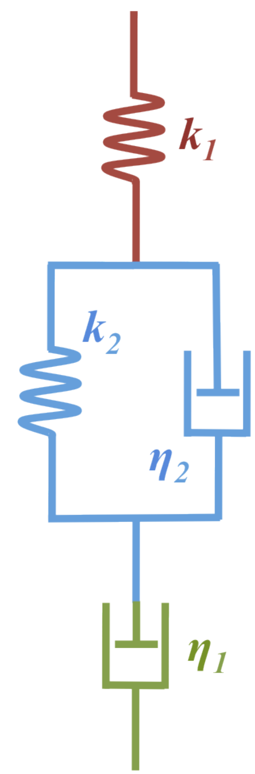
P. aeruginosa PAO1
50 μg/mL PMB

[Lu *et al.*, Soft Matter (2014)]

TIME-RESOLVED RESPONSE TO PMBN

- two-step response with delayed response for loss of integrity



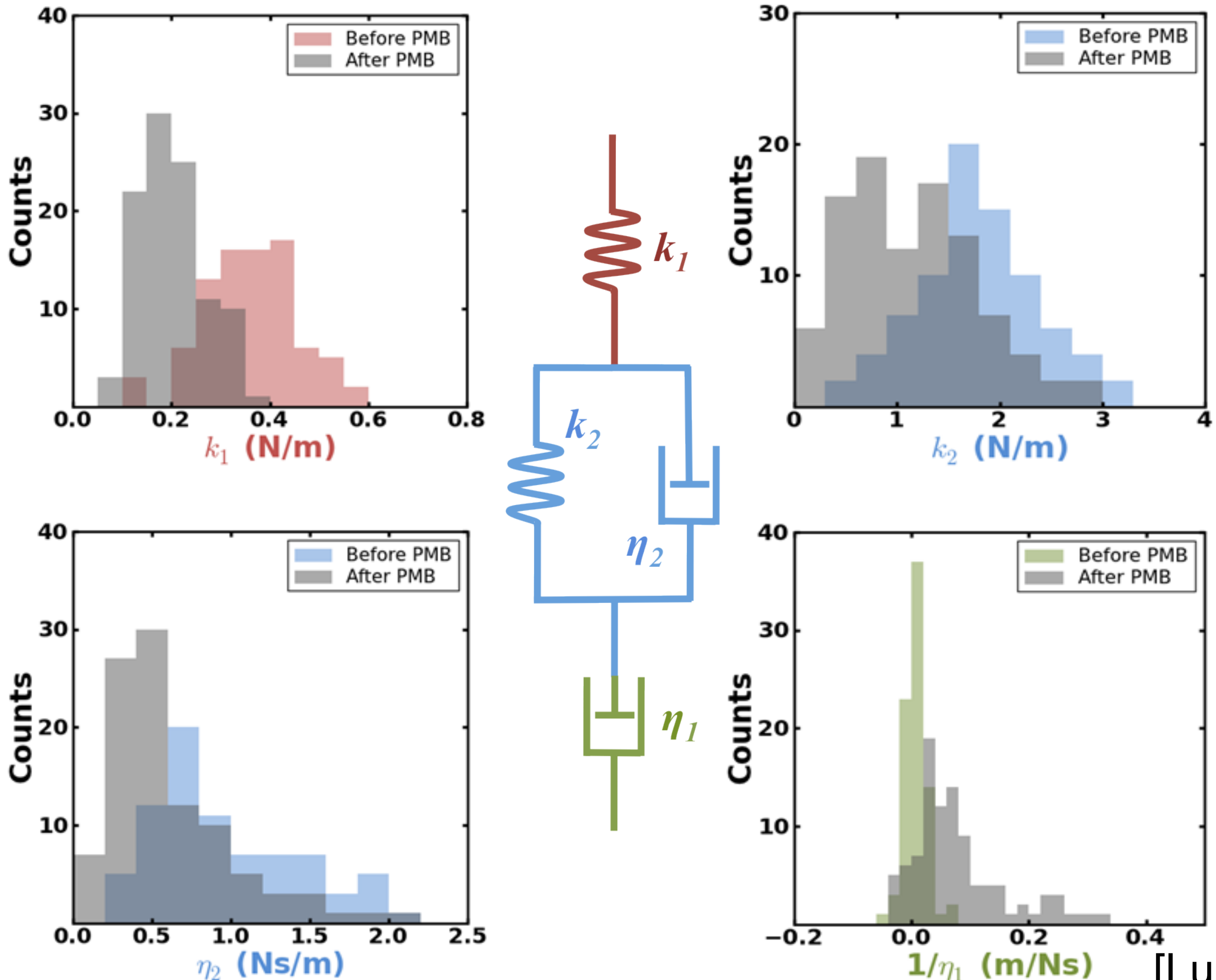


P. aeruginosa PAO1
50 μg/mL PMBN

[Lu *et al.*, Soft Matter (2014)]

EFFECT OF LOW PMB CONCENTRATION

- before & after 1 h exposure to 5 μg/mL PMB



P. aeruginosa PAO1
5 μg/mL PMB

- smaller number of compromised cells
- large decrease in k_1
- qualitatively different results consistent with different mechanism

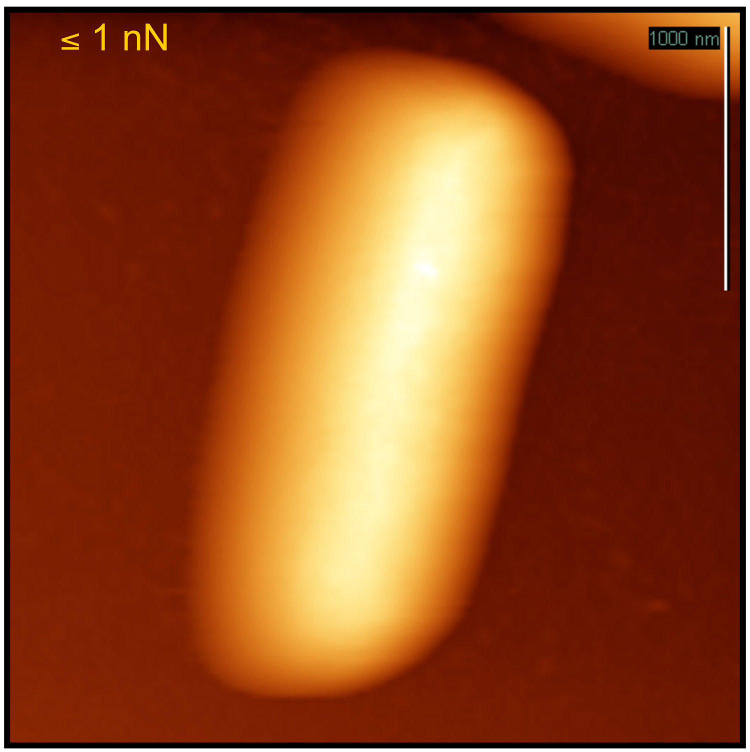
[Lu *et al.*, Soft Matter (2014)]

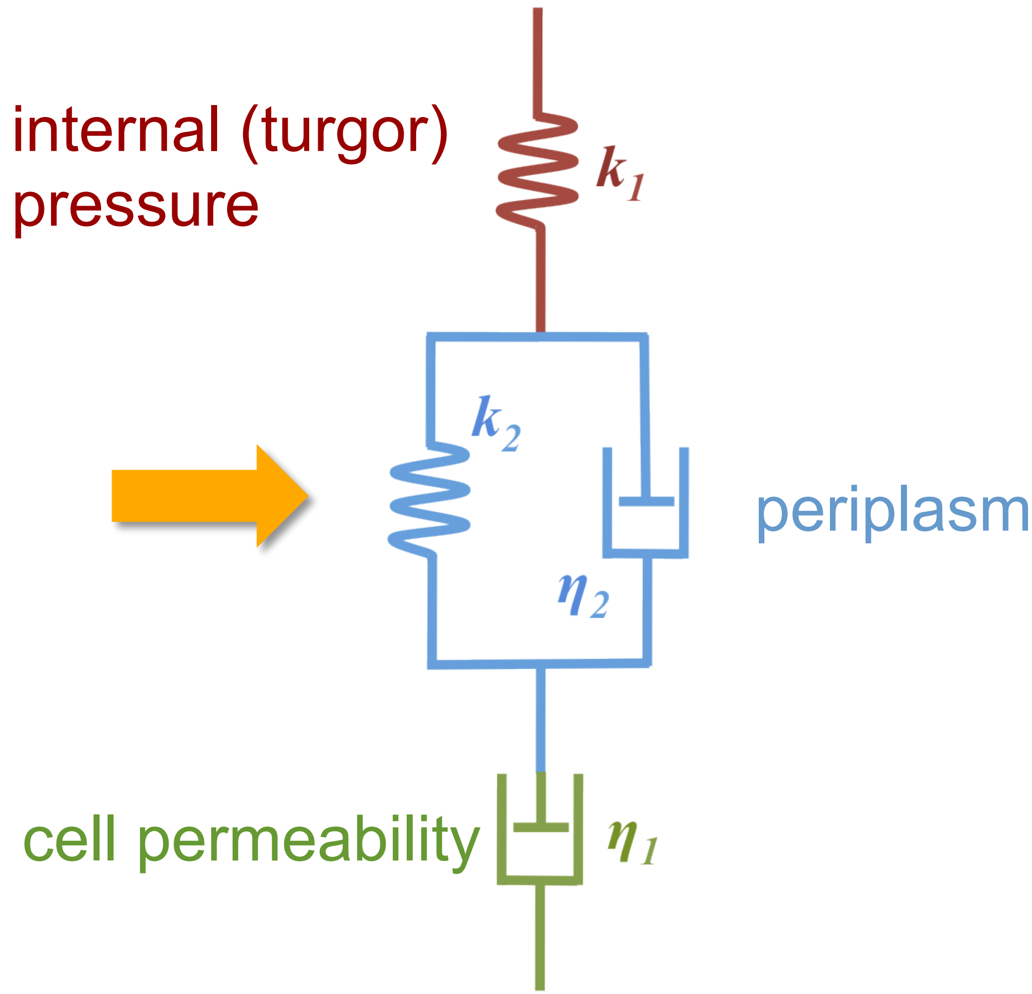
KEY RESULTS FOR PMB & PMBN

- $1/\eta_1$ provides measure of loss of integrity of cell envelope
  - smaller effect for PMBN
    - consistent with PMBN affecting only OM & PMB affecting both membranes → different mechanism of action
- abrupt changes to all parameters after certain time of exposure
  - suggests the existence of critical concentration
  - more abrupt change for PMB exposure consistent with promoted uptake mechanism
- large decreases in k_2 & η_2 for both PMB & PMBN
  - less elastic & less viscous → more water-like response
    - consistent with periplasm becoming more diluted

VISCOELASTIC MODEL OF CELL

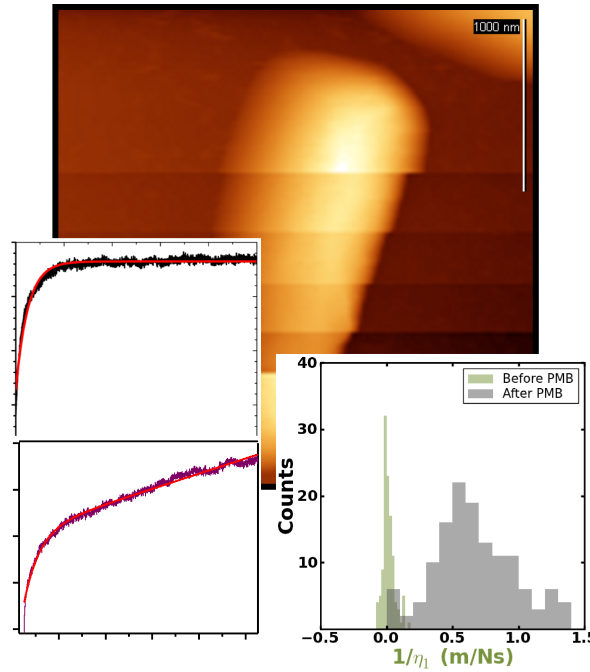
- can provide physical interpretation of phenomenological parameters

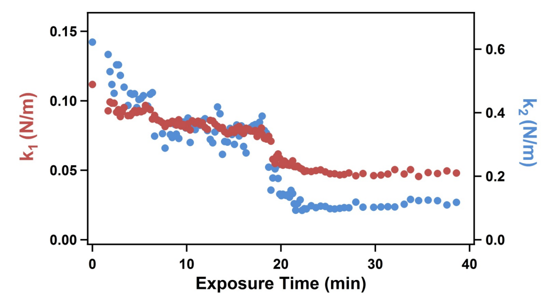




SUMMARY

- viscoelastic properties of bacterial cells
  - AFM creep deformation experiment is an *in situ*, reliable measure of mechanical response
  - effect of cationic peptides
    - distinct differences for structurally similar compounds
      - insight into mechanisms of action





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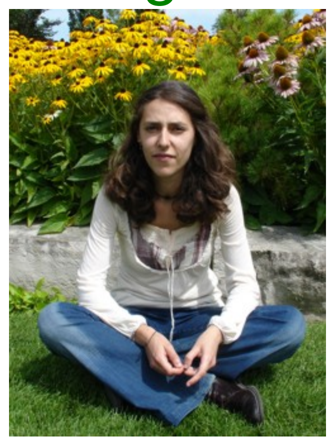
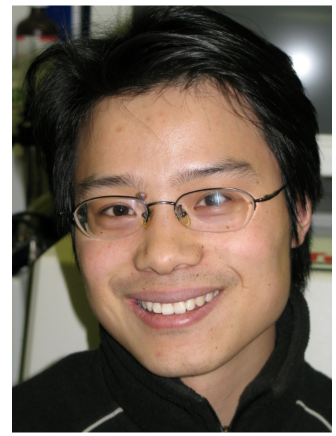


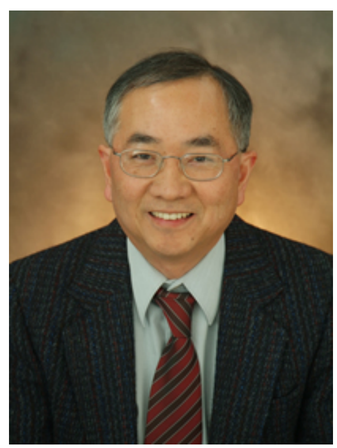
Shun



Grant

Richard

Joe



Peter

Sarah