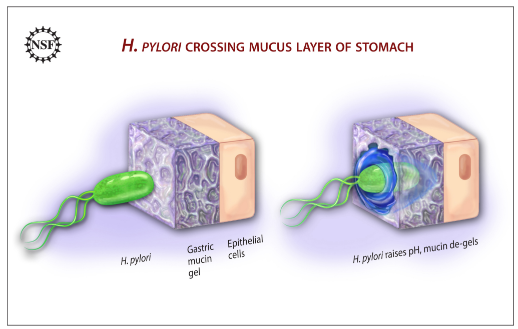
Gelation of mucin glycoprotein: Protecting the stomach from autodigestion

Rama Bansil
Boston University



Gastric Physiology

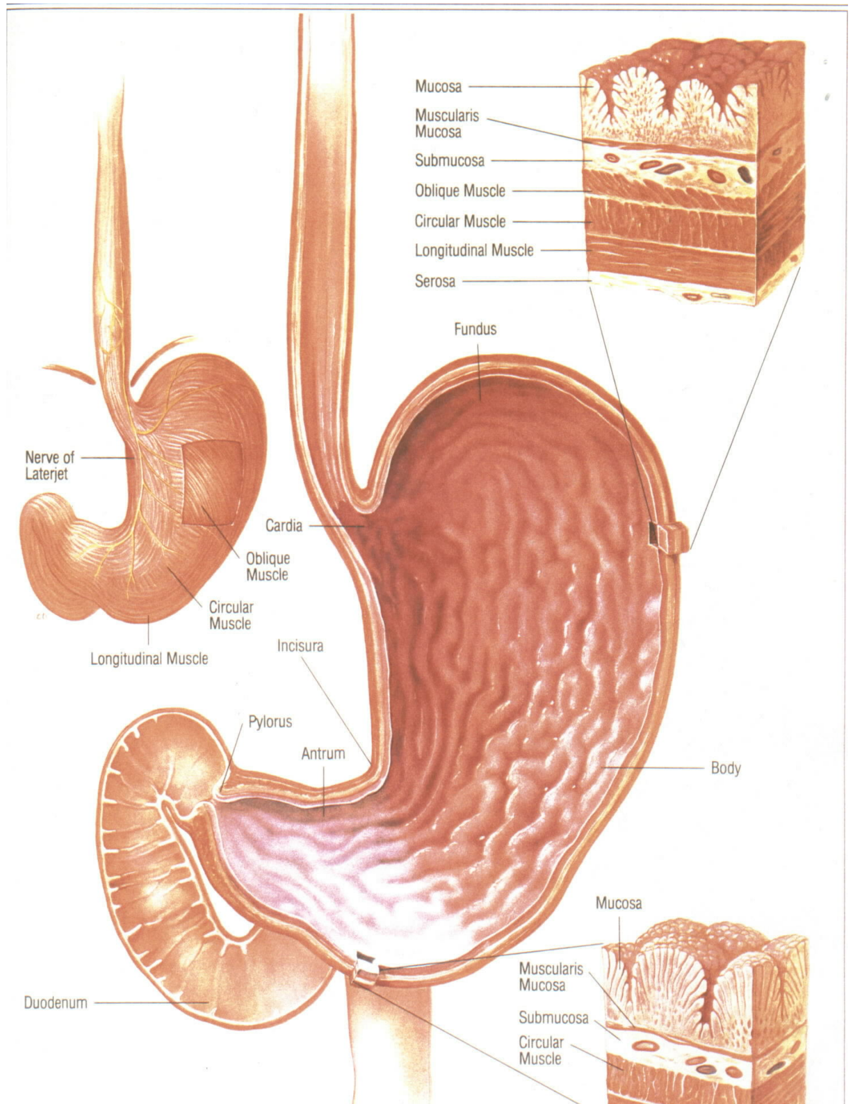


Illustration from Peptic Ulcer Disease, ed P.B.Molinoff 1990

Gastric Mucus: Home of *H. Pylori*

pH 2

Decreasing pH

pH 7

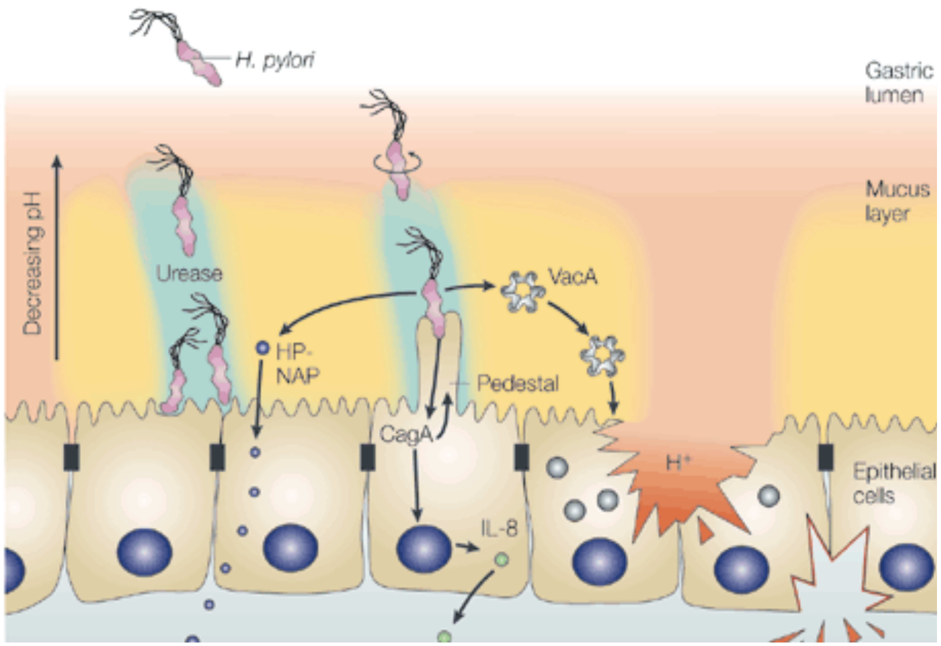


Image Credit: *C Montecucco and R Rappuoli* LIVING DANGEROUSLY: HOW *HELICOBACTER PYLORI* SURVIVES IN THE HUMAN STOMACH. NATURE REVIEWS: MOLECULAR CELL BIOLOGY **2** JUNE 2001, 457

Helicobacter Pylori (H. Pylori)

- Causes ulcers
- Spiral shaped
- Multiple flagella
- Swims through gastric mucus
- To survive at low pH it secretes urease to raise pH: $(NH_2)_2CO + H_2O \rightarrow CO_2 + 2NH_3$

Matrix (Gastric Mucus)

- contains a polymer Mucin →
- Viscoelastic properties: pH dependent

Gastric Gland

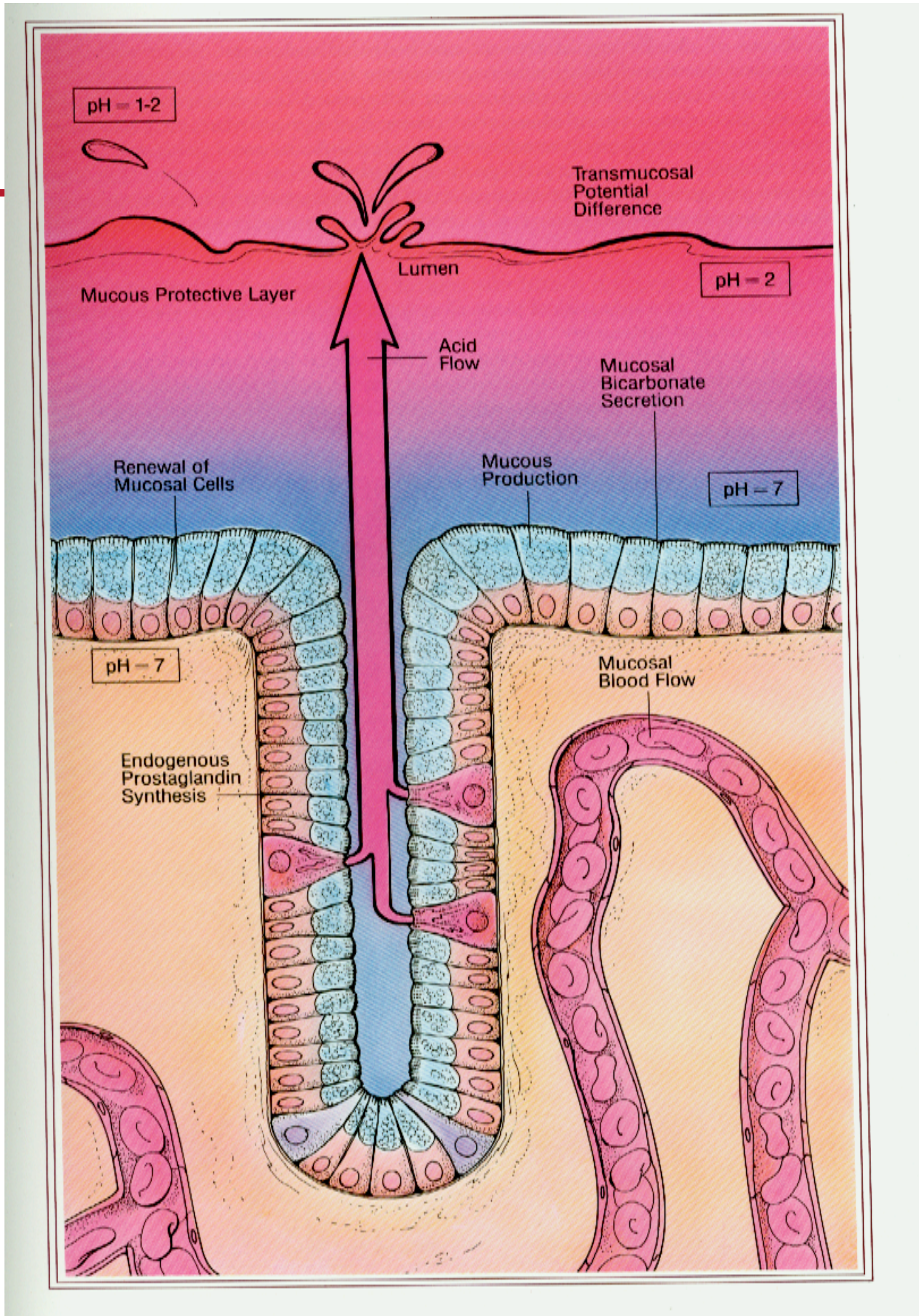
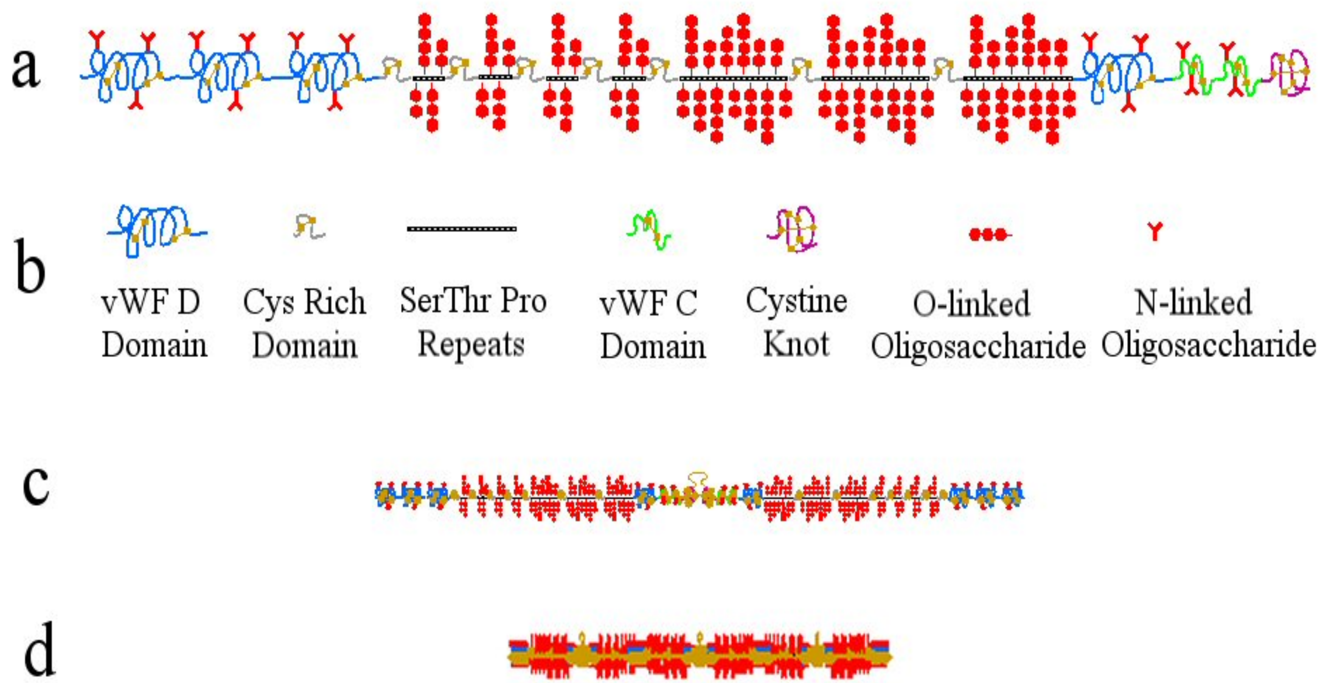


Illustration from Peptic Ulcer Disease, ed P.B.Molinoff 1990

Mucin Glycoprotein

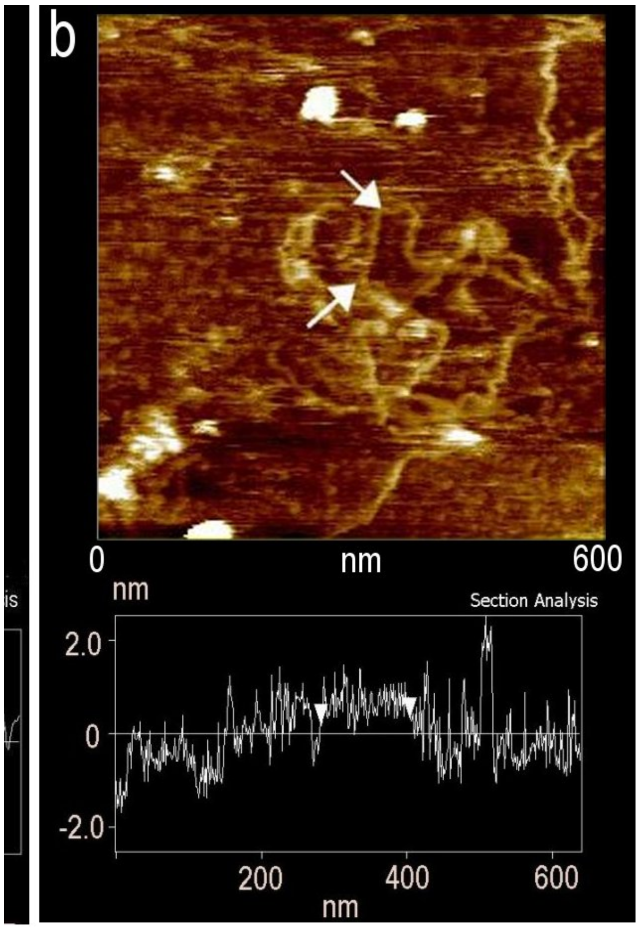


Mucin -- High MW (0.5-20 Million) polymeric glycoprotein

Covalently grafted oligosaccharide brush on STP tandem repeats
Subunits linked via S-S bonds
Multiblock copolymer—alternating polyelectrolytic brush (hydrophilic) –slightly hydrophobic block

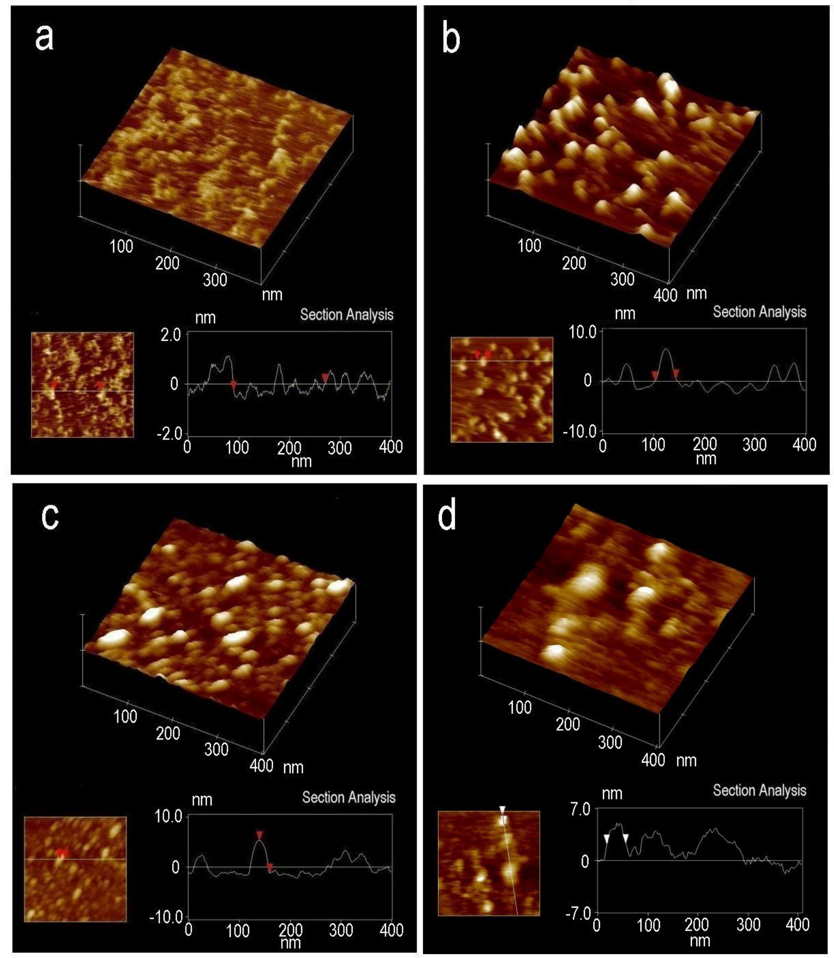
AFM images of mucin molecules at different pH

(b) pH 6



pH 6

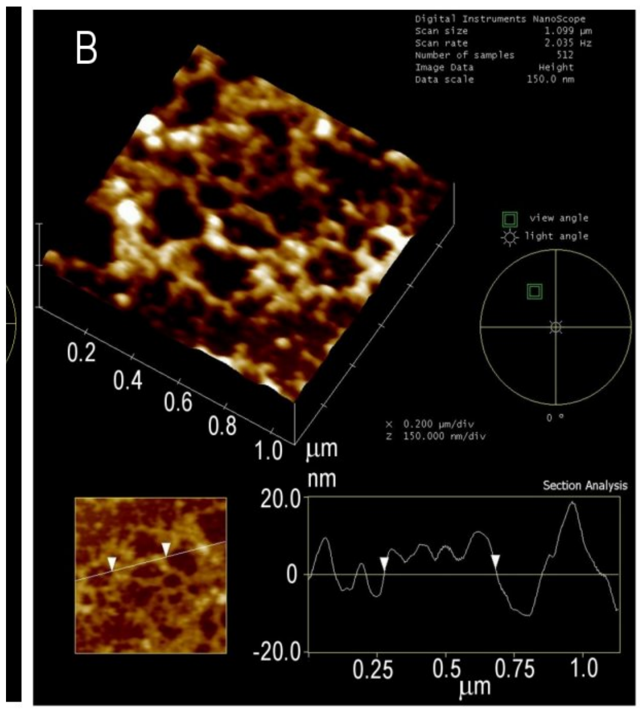
(a) pH 5 (b) pH 4



pH 2 deglycosylated

Z Hong, B Chasan, R Bansil, B Turner, K R Bhaskar, N H Afdhal, Biomacromolecules, 6, 3458, 2005.

Mucus

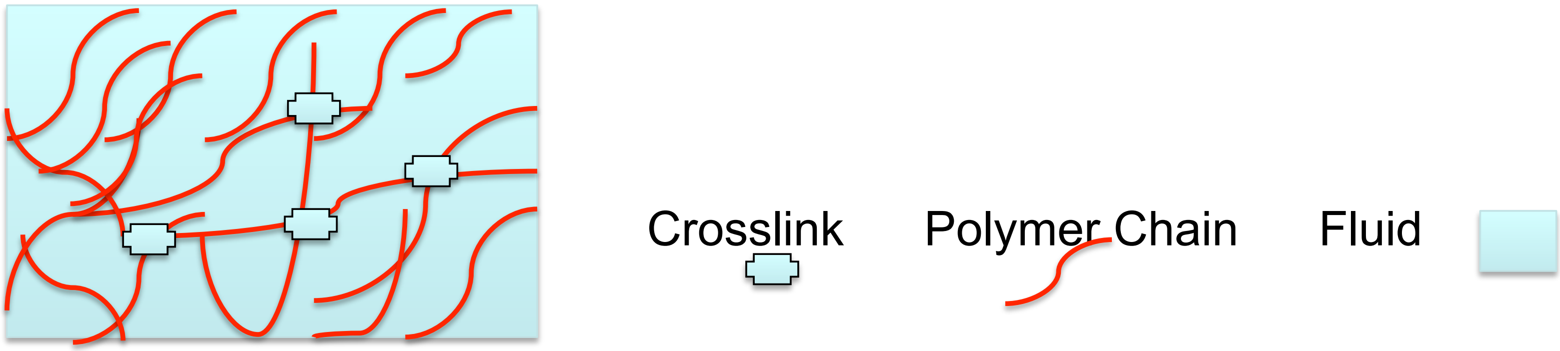


Mucus: Water (95%) + **Mucin Glycoprotein** (gel forming viscoelastic component of mucus, 3%) + other small molecules

Human mucus gel at pH 2. (A) 3 µm scan showing a clear network with well-defined pores. (B) A 1µm x 1µm region of the image shown in A is magnified revealing a “pearl necklace” morphology.

Z Hong et al. Biomacromolecules, 6, 3458, 2005.

What is a gel?



Gel—Crosslinked polymer immersed in a fluid

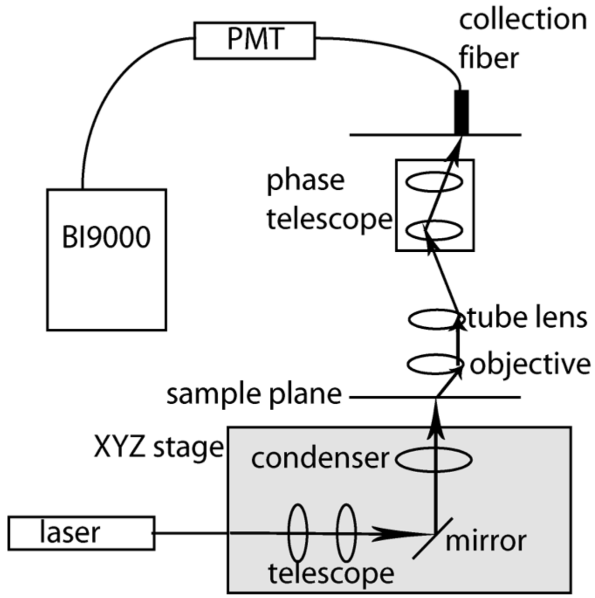
Increasing extent of crosslinking reaction p, critical transition at p_c

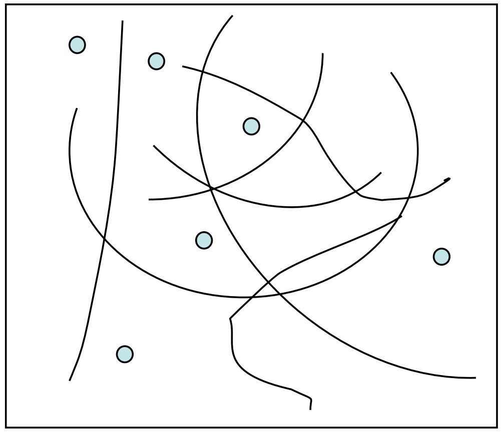
Sol—finite linear and branched molecules

Gel - infinite molecular weight, sample spanning network

$p \rightarrow p_c$ Viscosity diverges $\rightarrow \infty$ at critical extent of reaction, onset of elasticity at $p \geq p_c$

Microrheology with tracer particles





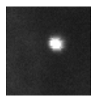
DLS---Measure Intensity correlation function, obtain diffusion and elastic constant and mesh size

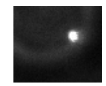
Microscopic particle tracking—Directly measure Brownian motion $<r^2(t)>$

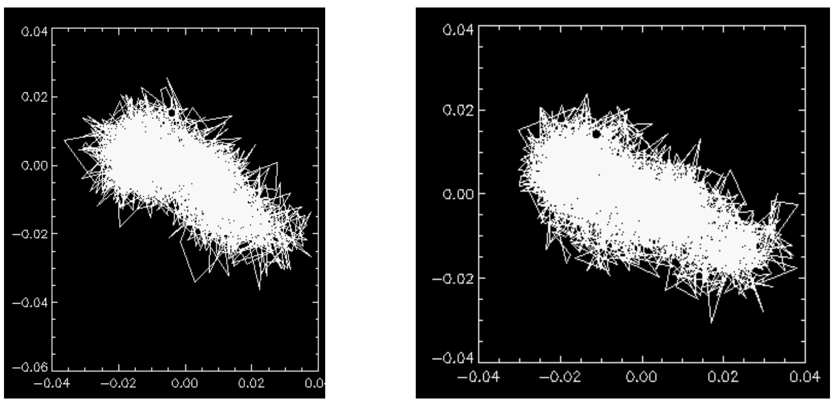
Heterogeneity in pH 2 PGM

Most beads are stuck in pores of the gel:

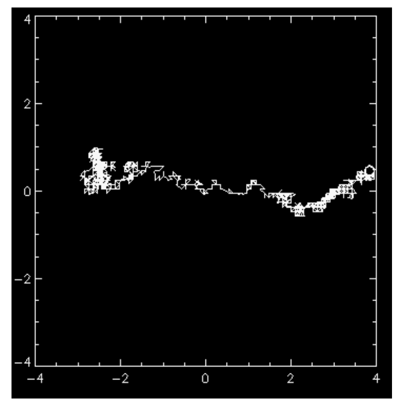
Some beads end up in regions which allow higher local mobility – i.e. a large channel in the gel





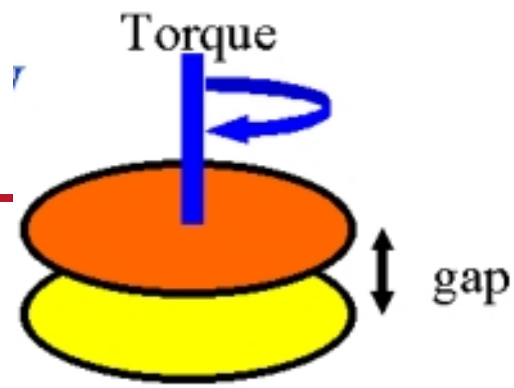


Trajectories for stuck beads



Trajectory of a bead moving through a channel

Rheology Experiments



- Stress Sweep – Vary applied oscillatory shear stress: G' and G'' vs σ
- Frequency Sweep – Vary frequency of oscillatory shear: G' and G'' vs ω
- Flow Test – Step increases of steady shear rate (or stress): η vs $\dot{\gamma}$
- Creep Test – Step Stress applied and released

$$J(t) = \gamma(t)/\sigma_0 \text{ vs } t$$

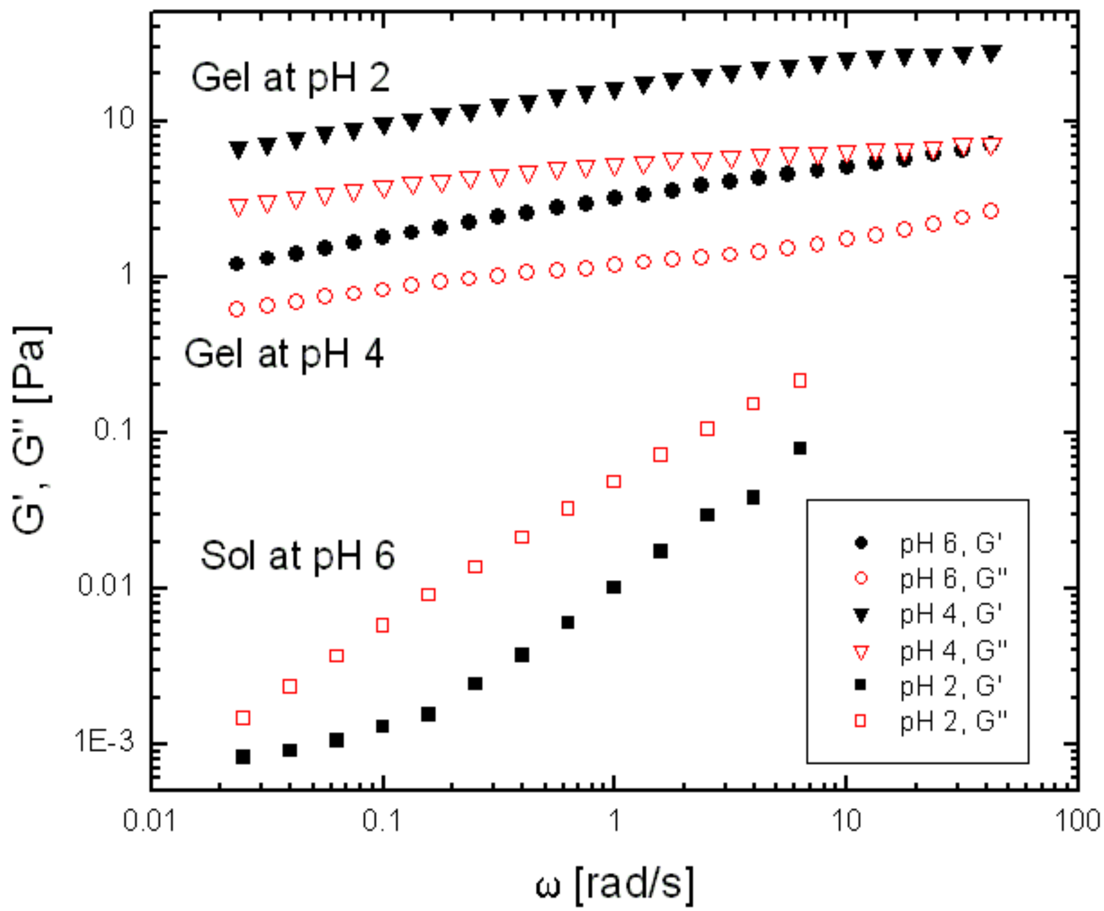
G' = elastic (storage) modulus

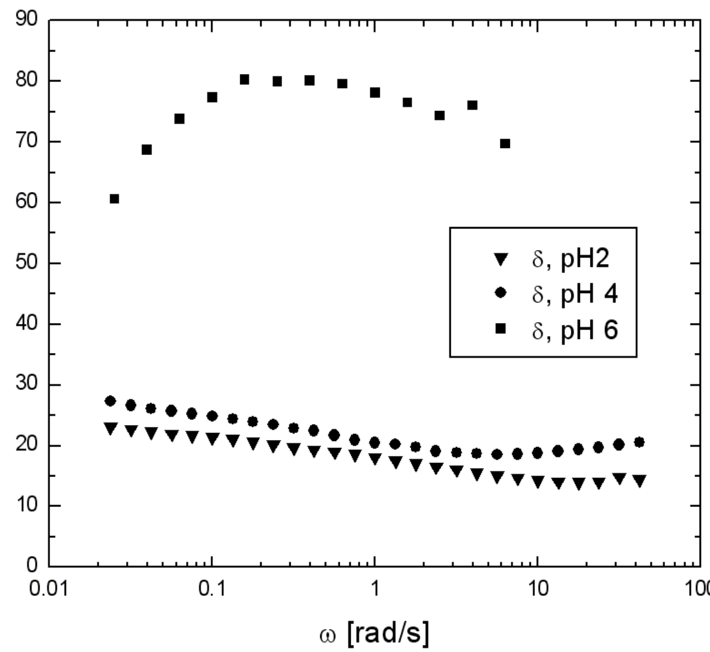
G'' = viscous (loss) modulus

TA Instruments AR-G2 Bulk Rheometer

All experiments on 15 mg/mL PGM buffered with 10mM phosphate-succinate buffer

pH-Dependent Gelation





G′ = elastic (storage) modulus

G′′ = viscous (loss) modulus

tan(δ) = *G′′* / *G′*

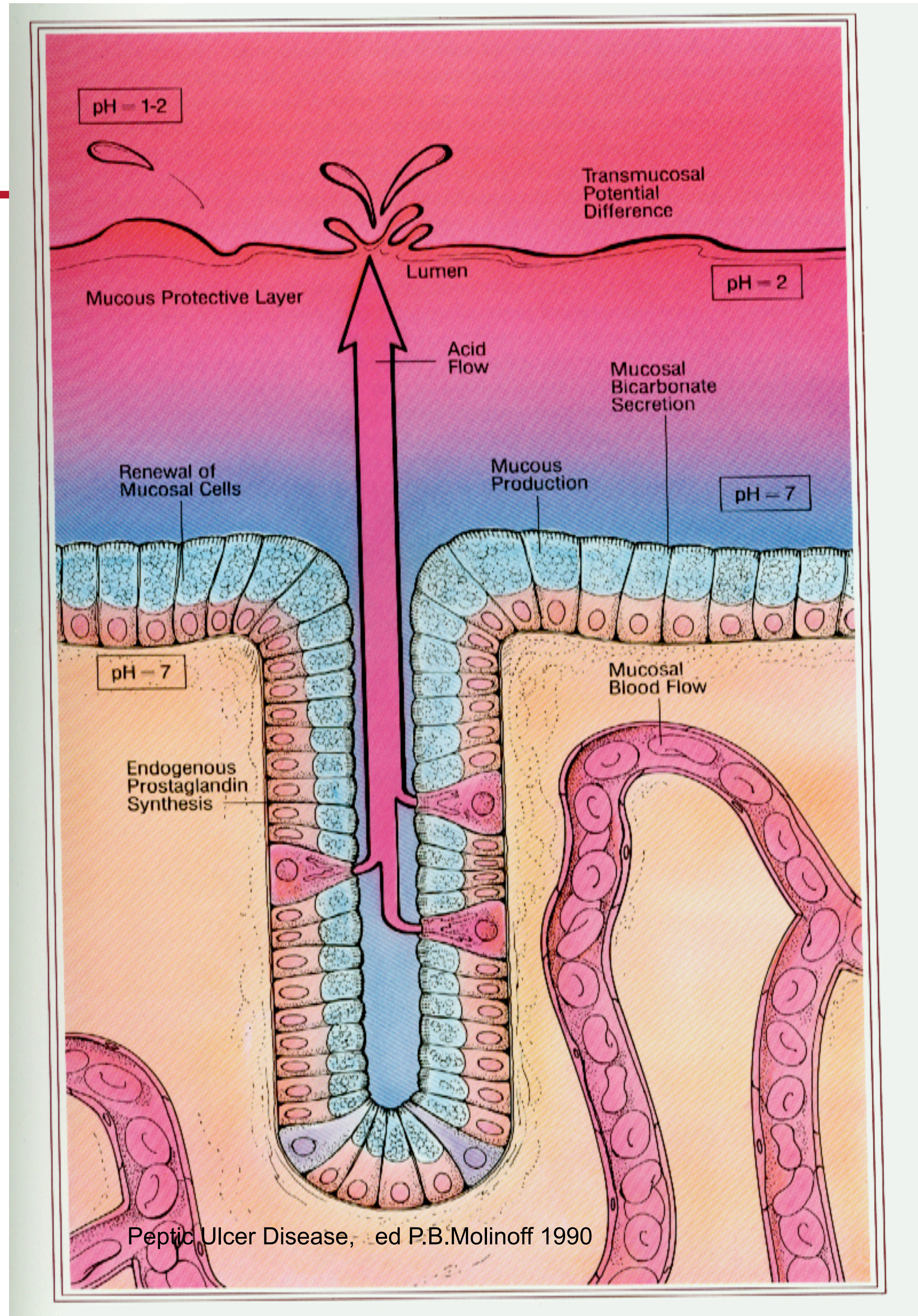
δ > 45: sol

δ < 45: gel

Celli, Turner, Afdhal, Ewoldt, McKinley, Bansil, Erramilli-- Biomacromolecules 8, 1580, 2007

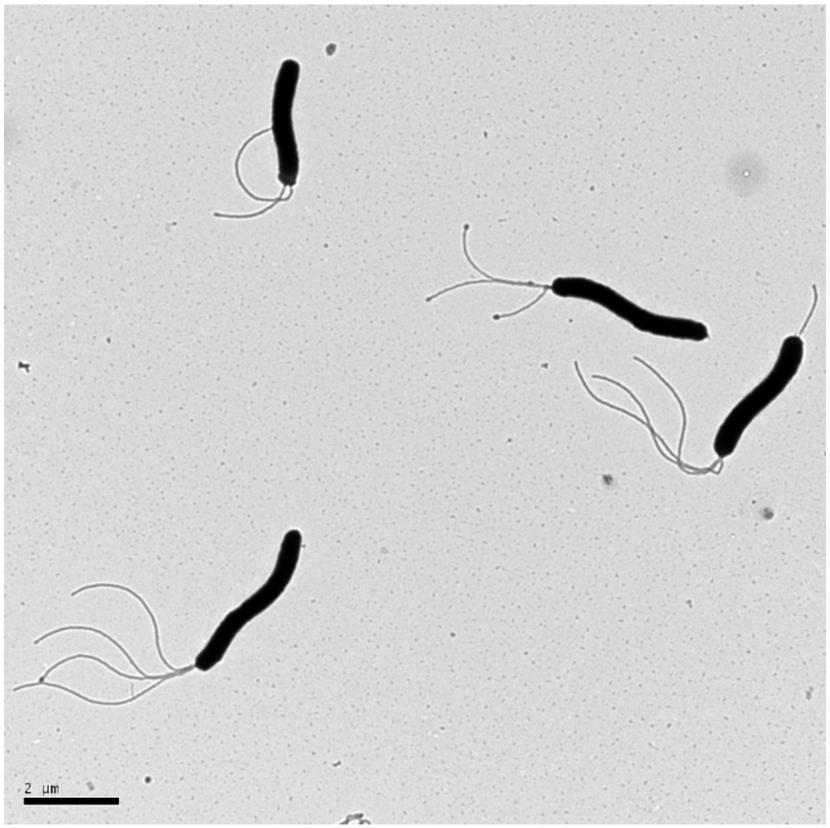
Gelation can protect the stomach from autodigestion

- Acid flows as a jet (viscous finger)
- Mucin surrounding the acid finger gels--forming a viscoelastic "pipe"
- Gland stops firing--pH rises, gel--> sol "pipe disappears"
- Surface layer of mucus stays gelled while stomach is acidic--preventing backflow



Peptic Ulcer Disease, ed P.B.Molinoff 1990

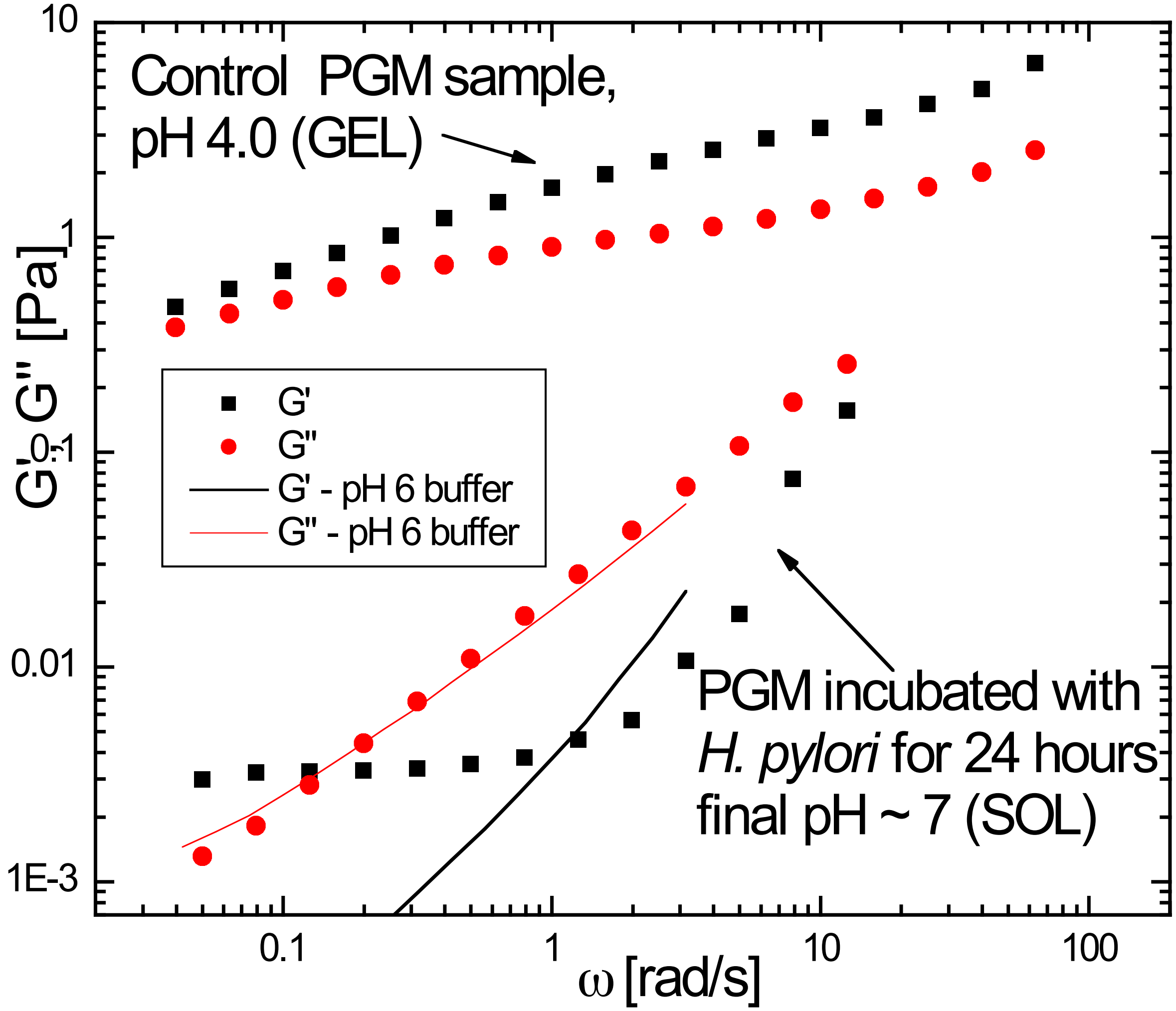
TEM images H. pylori



- How does H Pylori affect the structural and mechanical properties of mucus and mucin?

- Are the underlying motility mechanisms fundamentally different in a visco*elastic* fluid as compared to swimming in a purely viscous environment?

How does *H. pylori* affect Mucin Rheology



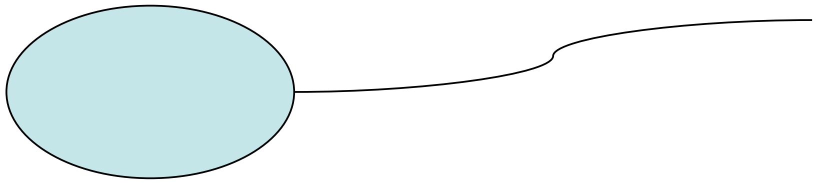
- PGM + 5 mM urea + ATCC strain 43504 *H. pylori*
- pH initially depressed by addition of HCl
- Final PGM conc = 15 mg/mL

H. pylori* induces gel to sol transition; directly correlated with pH elevation due to *H. pylori

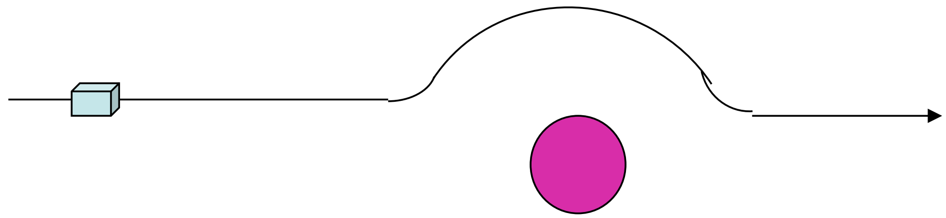
Celli et al, PNAS August 25, 2009 vol. 106 no. 34 14321-14326

Background on Swimming of Bacteria

- Swimmer feels drag forces F_{drag} due to the hydrodynamics of the water
- Stokes Law $F_{drag} = -\Upsilon v$
- Υ is friction coefficient (depends on shape and size of swimmer and viscosity of fluid)
- Einstein--Diffusion Coefficient $D = kT/\Upsilon$



Low Renolds number flow



Streamline of fluid going around an obstacle of diameter d

- ρ=fluid density, η= viscosity of fluid
- V= speed of fluid flow (far away from obstacle)
- d=diameter (size) of particle

  (cube of fluid volume l^{3}, area of a face A~ l^2)
- Inertial term =ma~ ρ l^3 v^2/d
- Friction force on one face = - η A(dv/dx): net f~ ηl^3 v/d^2
- Inertia/Friction~ ρVd/η =R (Reynold's number)
- Small R – friction dominates—

  inertial forces <<viscous forces

Some typical magnitudes of R

- In Water
- η / ρ (kinematic viscosity) = 0.01 cm^2/s

E coli d~ 1.5 μm, v= 30 μm/s R = 0.5 $x10^{-4}$ <<1

Protein d ~6 nm, v=8m/s R=0.05

friction dominates

goldfish d~10 cm, v=10 cm/s, R = 10^4

swimmer d~ 2m, v=1m/s, R = 2x 10^6

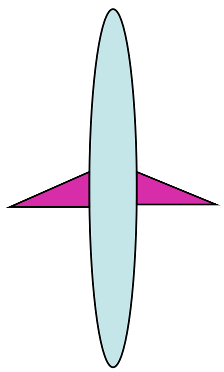
inertia dominates

- Goldfish swimming in molasses would feel friction
- A marble pulled through corn syrup, force < 0.03 N friction dominates

- Turbulence

- R < 2000 Laminar, R> 3000 Turbulent, 2000<R<3000 unstable
- Low R --- no turbulence

Mechanisms of swimming



Possible solutions:
bending of arms
(cilia)

Corkscrew like motion
of tail (flagella)

Moving “arms back and forth
without bending

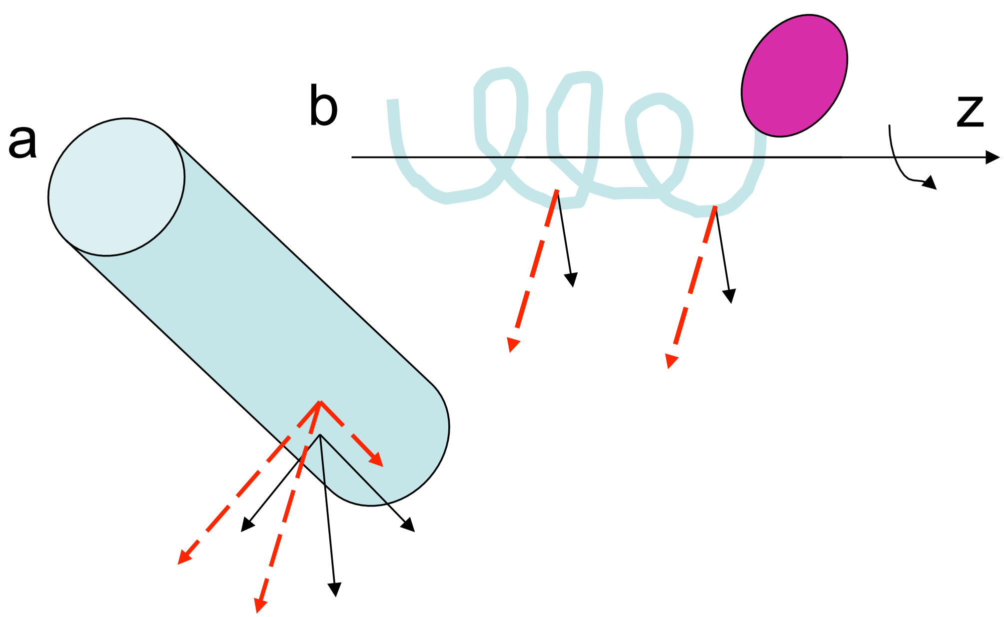
can propel forward by pushing
water back.
Move at constant speed:
force exerted = drag force

drag force is always opposing
direction of motion, forward and
backward motions along same line.

Since arms move back by same
amount to return to original position
forward and backward
displacements will cancel out.

Cork screw rotation of flagella

Bacterial Flagella is a stiff, thin, helical fiber attached to the body at one end—like a phone cord connected at one end—cranking the flagellum counterclockwise will move the bacterium to right



(a) Drag coefficients different along axial and radial directions

$\gamma_{\parallel} < \gamma_{\perp}$: $f_{\parallel} < f_{\perp}$

$f_{\parallel}$ is opposite $v_{\parallel}$, $f_{\perp}$ opposite $v_{\perp}$

F net is not directly opposite v net

(b) helical tail cranked counterclockwise—net force along z-direction, needs a left force to spin in place,

but one end of helix is not fixed, so cannot apply the leftward force.

So, Net force to the right.

Net motion is like a corkscrew

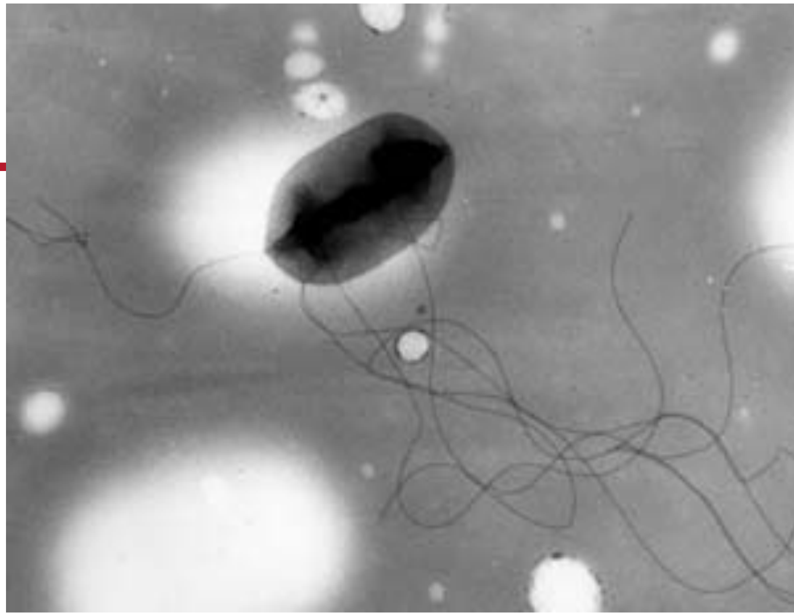


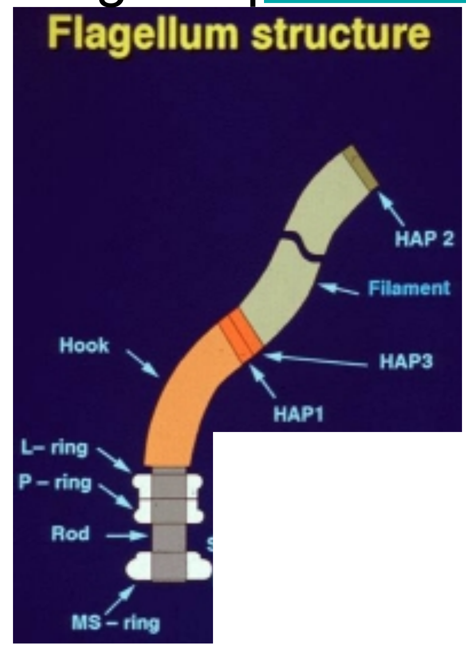
Image provided by Elizabeth White.
Courtesy of the Centers for Disease Control and Prevention.
Doc Kaiser's Microbiology Home Page

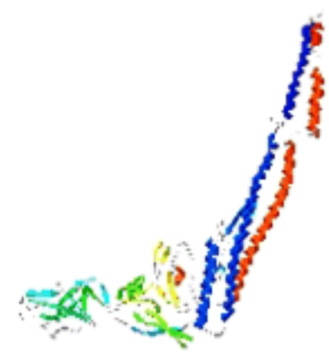
Flagella ~ proton pump

5 µm long helix, 11 protofilaments

40 kDa flagellin protein

Alun.Morgan@hri.ac.uk.





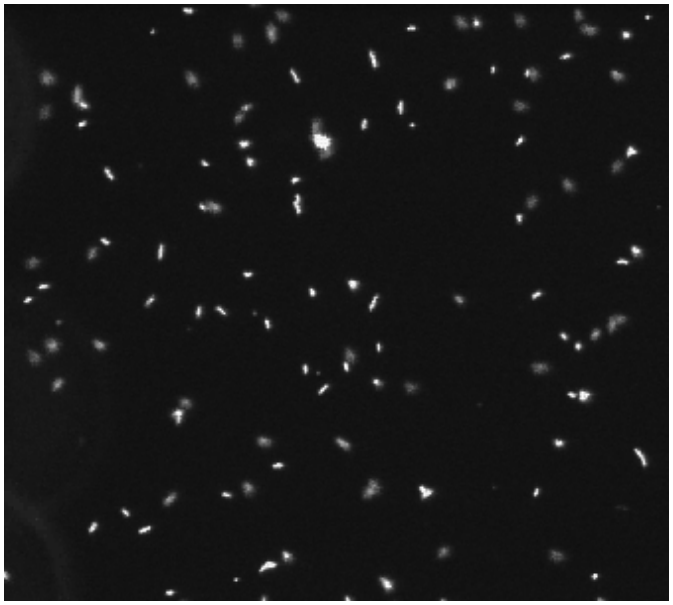
Brian Tripp, Western Michigan Univ.
http://homepages.wmich.edu/~btripp/index.htm

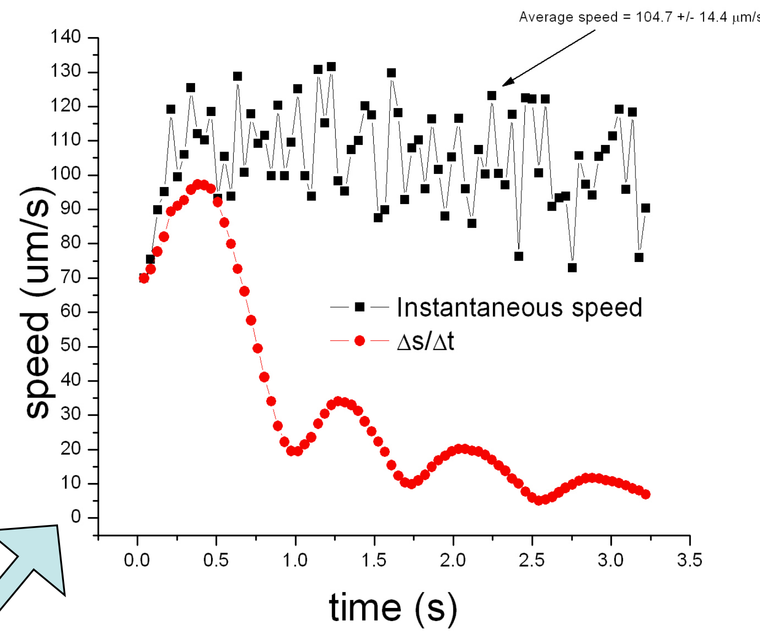
Bacterium moving in a straight line, all flagella form a bundle—move counterclockwise (viewed from rear of cell)—propel the bacterium forward.

Bundles come apart, bacterium tumbles about.

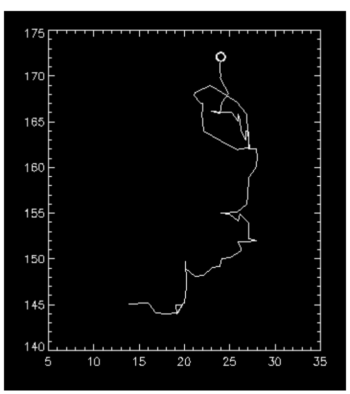
Bundles reform and bacterium moves off in another direction.

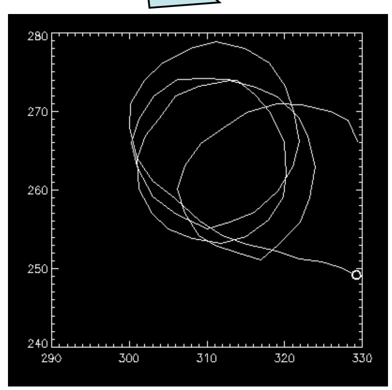
Bacteria Tracking

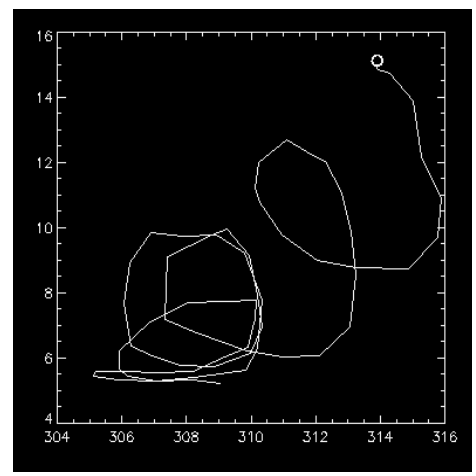




Above: Hp in culture broth. Darkfield

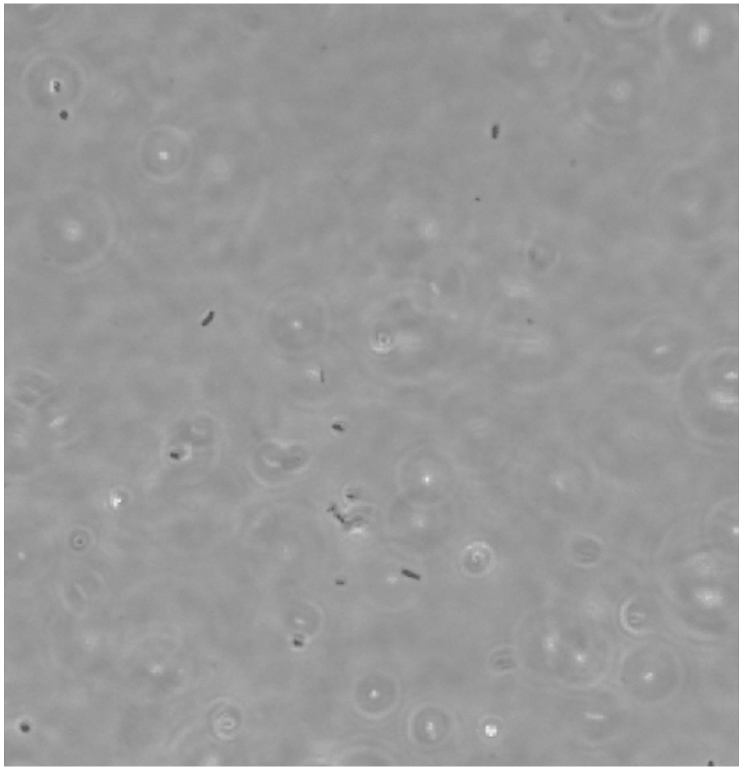




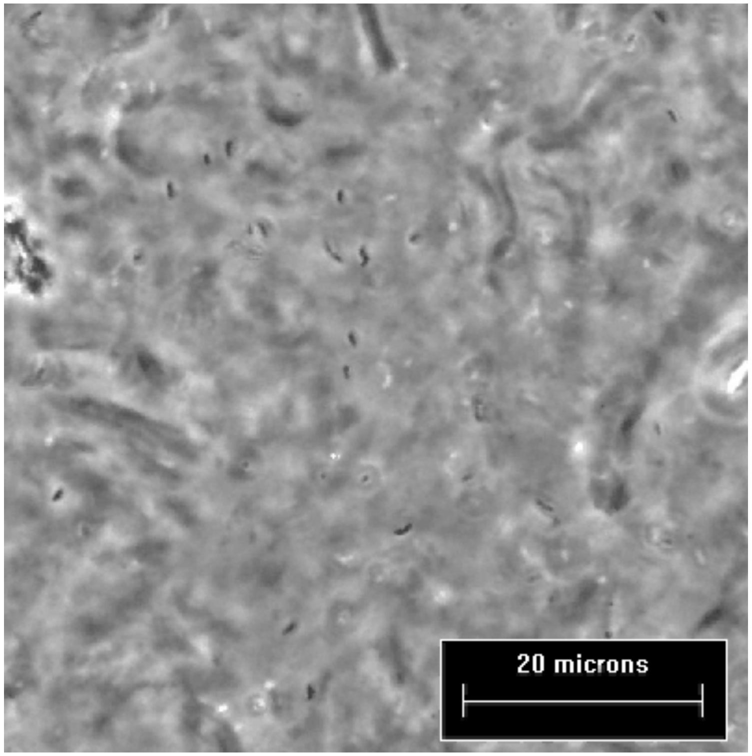


Some representative trajectories: left, middle, right: Tracks 40, 86, and 37 out of 577.

pH Dependence of Motility

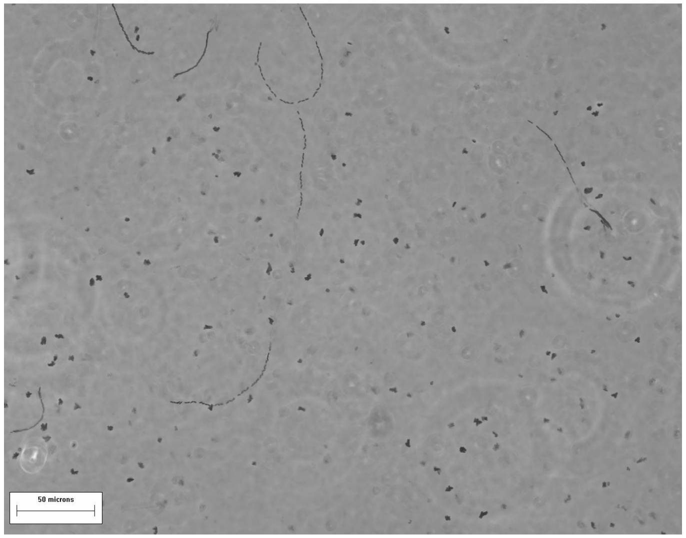


pH 6: Analysis shows that HP swim at velocities ~ 30um/s



pH 2: Motility is severely hindered by the viscoelastic gel

Motility is pH dependent



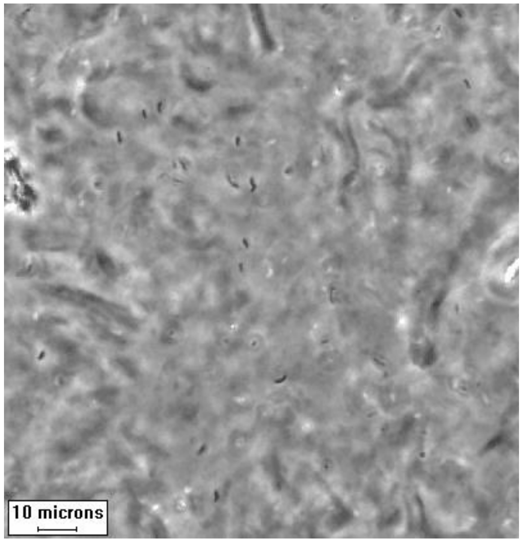
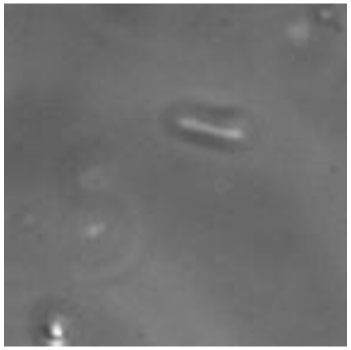
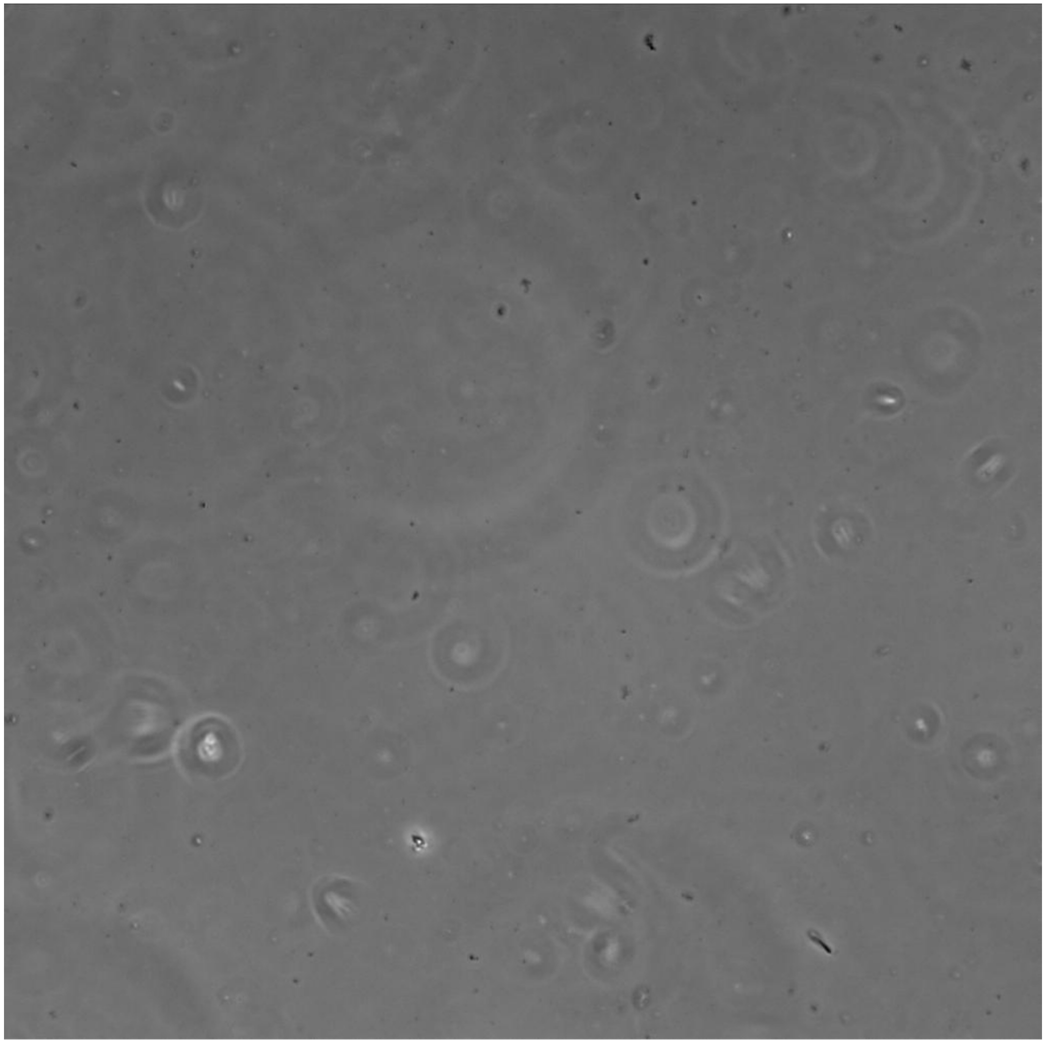


Figure 2. (Left) A series of frames from the movie of H. pylori moving in pH 6 are stacked to show the trajectories of several bacteria (large curved lines). The scale bar is 50 ì m, size of the image is 434 x 331 ì m. The average velocity was ~ 30 ì m/sec.
(Right) Typical image from one frame of a movie showing that H. pylori were stuck in PGM in a PBS buffer at pH 2, magnification 40X. (Several bacteria are visible as small, dark, elongated objects about 2-5 ì m in size). Local rotation of the bacteria was seen in the movie (not visible in this single frame).

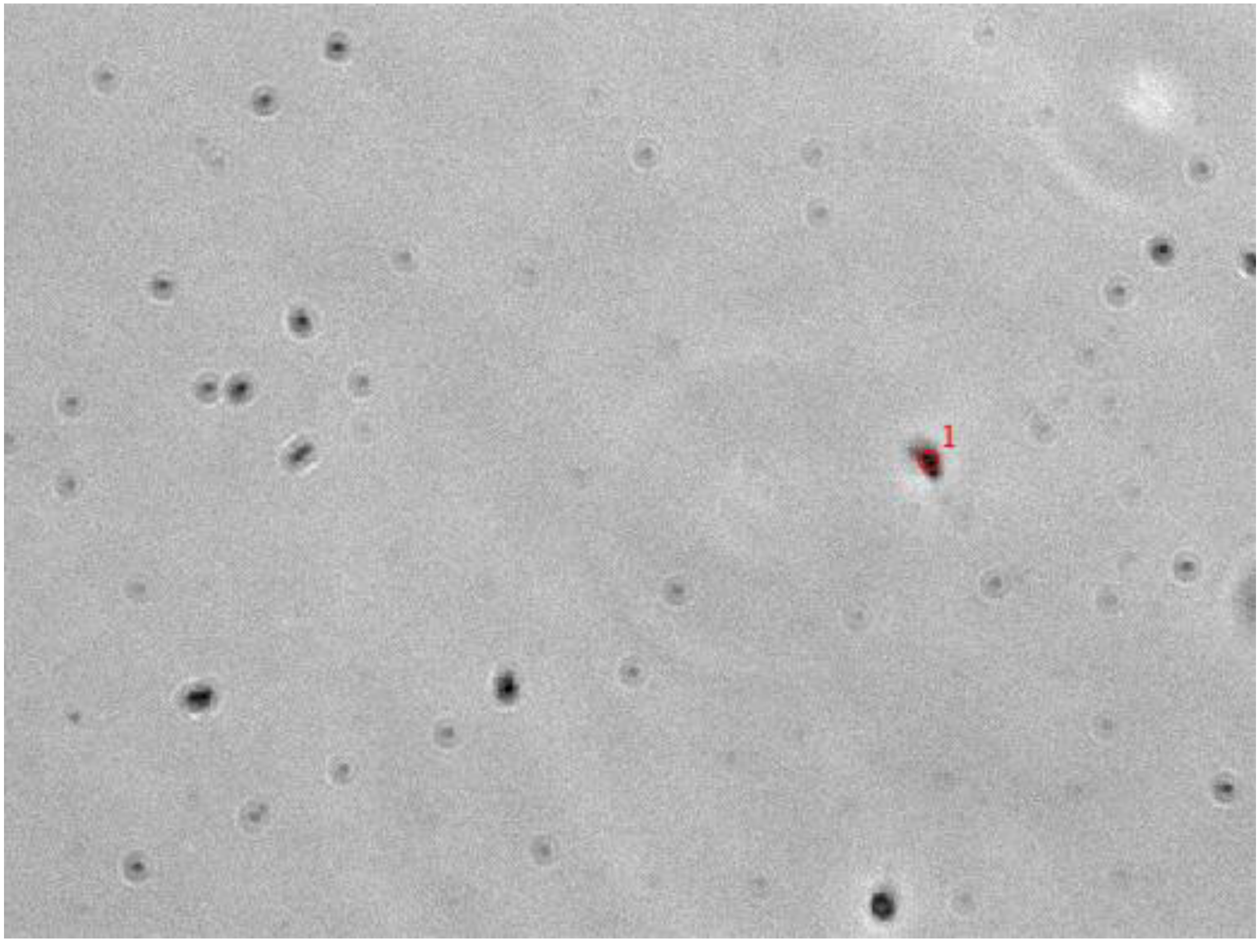
- HP07_031706-3_CROP_mpeg.avi

Wiggling/ Rotating bug

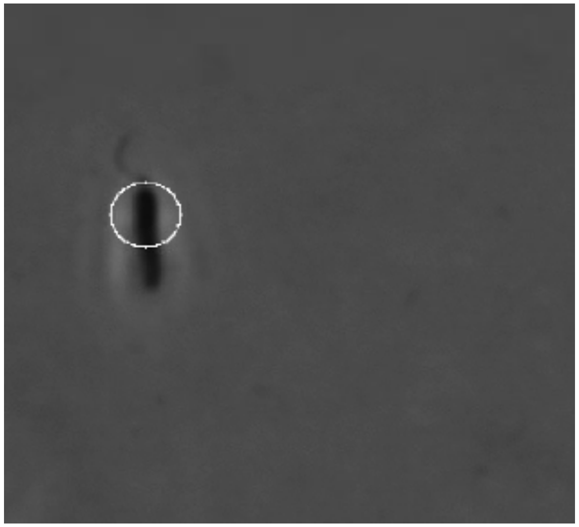




H. Pylori motility in pre gel solution



Movie of H Pylori flagella rotation at pH 4



- Flagella rotates in pH 4 PGM, but there is no translation

pH4_031706-3_features1.mpg

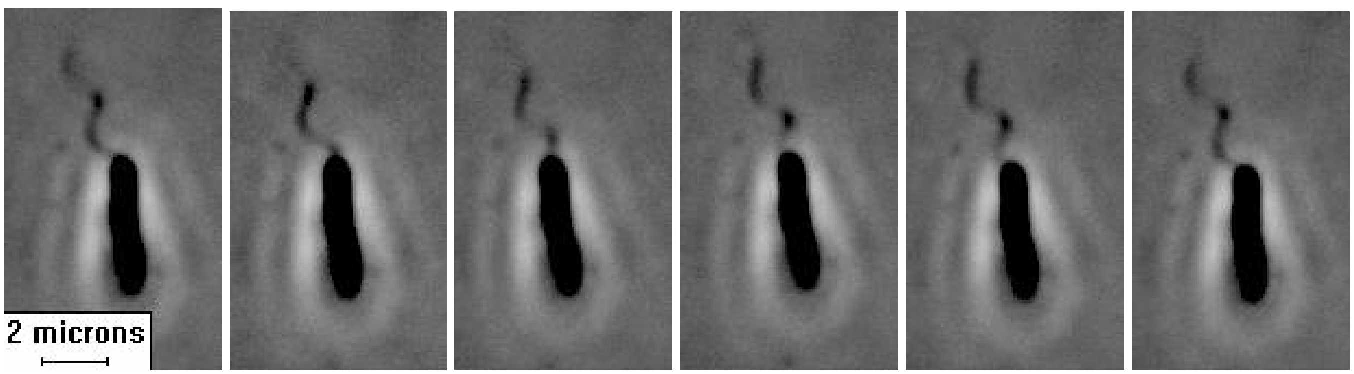
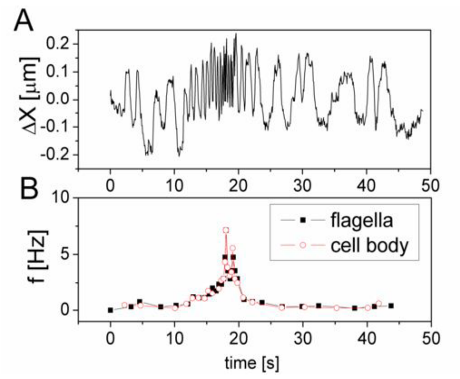
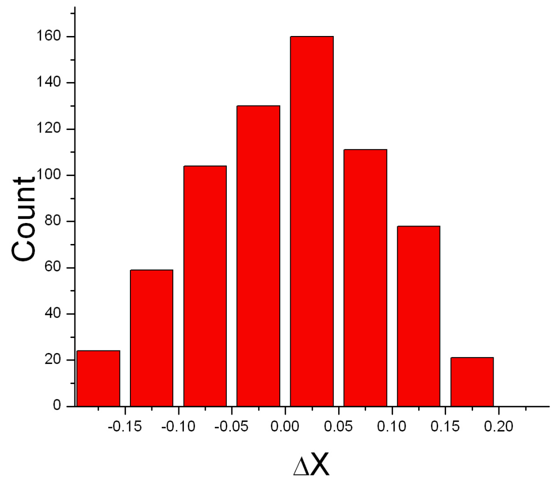


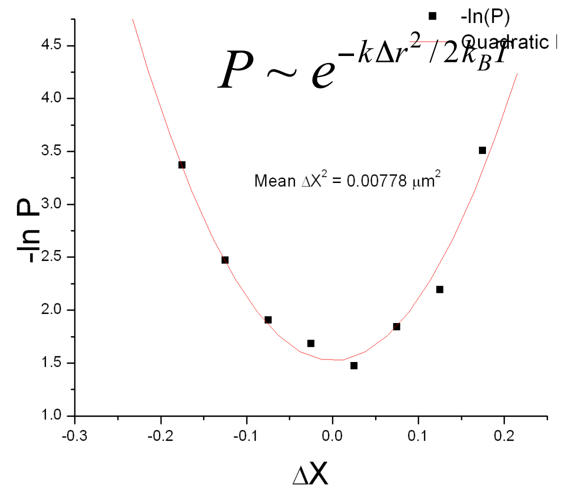
Figure 3. Phase contrast image from a H. pylori bacterium in pH 4 PGM shows flagellar rotation.

Celli et al, PNAS August 25, 2009 vol. 106 no. 34 14321-14326

Flagella motion





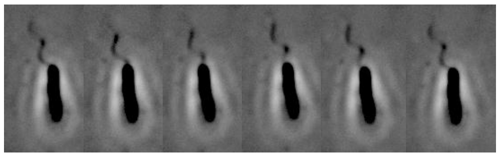


$$k = 4.9 \times 10^{-7}\ \mathrm{N/m}$$

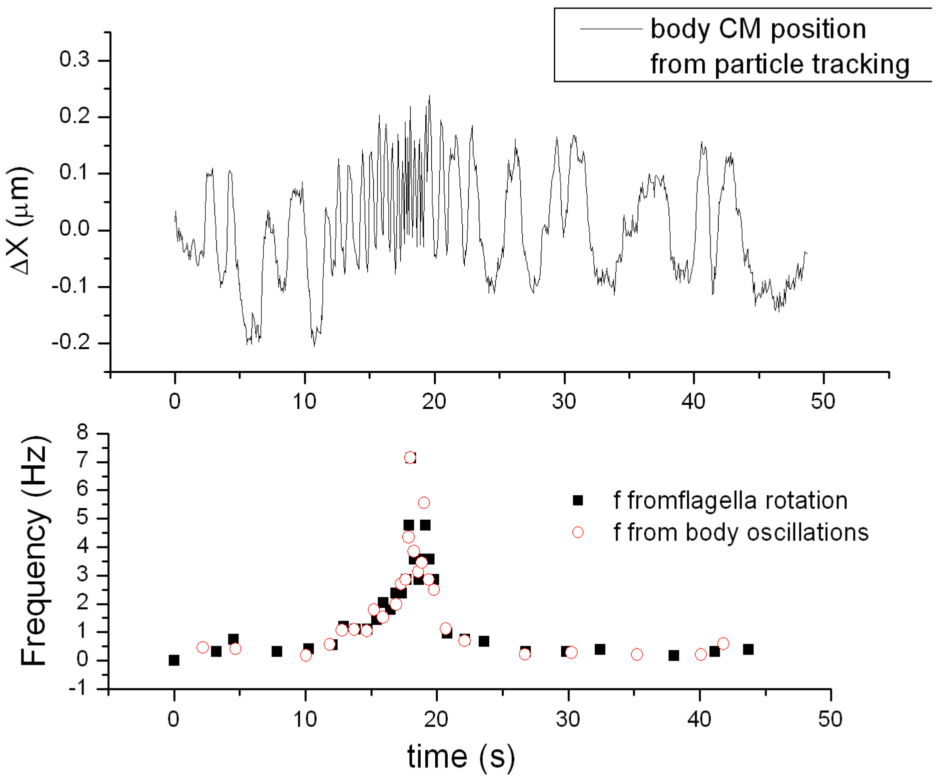
* *Similar to Analysis of Tang et al J Bacteriol. 187, 257-265 (2005)*

Celli et al, PNAS August 25, 2009 vol. 106 no. 34 14321-14326

Flagella Frequency



1. Count # of flagella cycles as function of time
2. Count body oscillations (from track data, upper part of graph) as a function of time

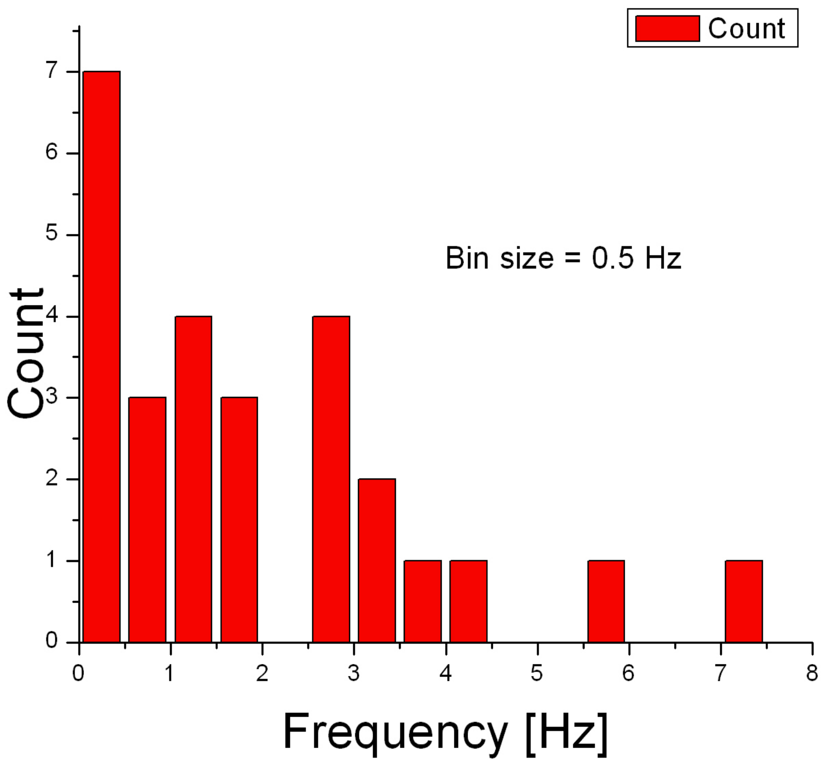


Frequency as a function of time – representative bacterium

How do body oscillations correlate with translational motility? Are body movements crucial to motility or incidental?

Also, what causes spikes in frequency? Breaking links in gel? Chemoattractant stimulation? Discrete molecular motors?

Flagella Frequency (2)



Is rotation frequency quantized and driven by disrete molecular motors as observed in *E. Coli* by Berg. et al?

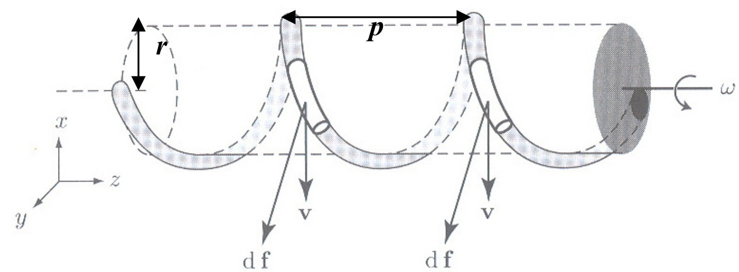
Histogram of flagella rotation frequencies (same bacterium as previous slide)

Motor Torque

$$T_m = \beta_f \omega_f - \gamma_f v_c$$

Hydrodynamic formulae – *Marigiyama et al.*
A more thorough analysis for this system would correct for shear thinning and elasticity…

$$\beta_f = \frac{2\pi\eta L}{\left[\ln(2p/d) - 1/2\right](4\pi^2 r^2 + p^2)}\left(4\pi^2 r^2 + 2p^2\right)r^2$$



From rheology and microscopy data:
$\eta \sim 1$ Pa-s
p= 2.1 μm, L = 3.17 μm and r =0.57 μm

→ $\langle T_m \rangle = 3.6 \times 10^{-18}$ Nm

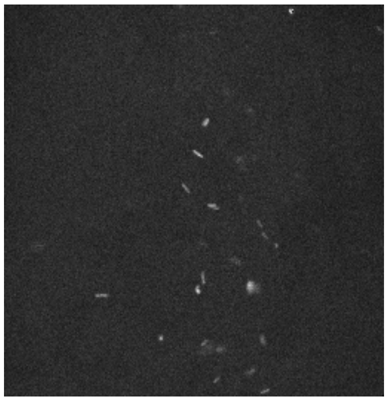
Comparisons:
E. Coli (Reid et al.):
$T_m = 1.3 \times 10^{-18}$ Nm
C. Crescentus (Tang et al.):
$T_m = 3.5 \times 10^{-19}$ Nm
(known to have low T_m)

How does this torque relate to a yield stress of 10 Pa?
Swept volume, $V \sim \pi r^2 L \sim 3\ \mu m^3$
Dimensional Analysis: $\tau \sim T_m / V \sim 1$ Pa

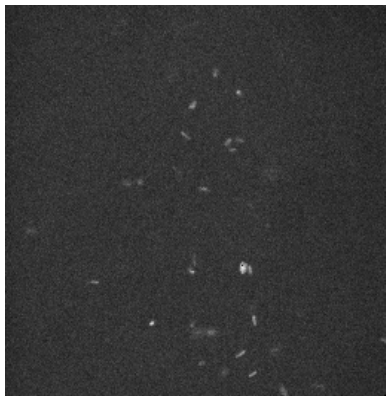
Analysis of motor torque based on cell body rotation, $T_m = \beta_c \omega_c$
…less robust, but yields results within factor of 3

Two-Photon Fluorescence

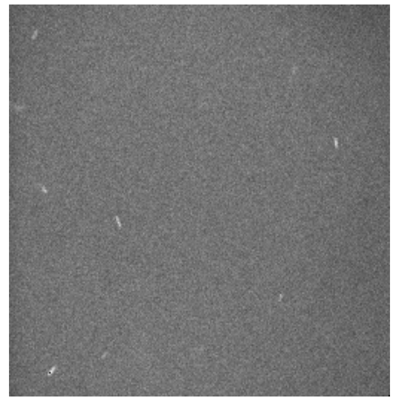
Two-photon fluorescence microscopy of PGM with pH-sensitive dye (BCECF) with 5 mM urea present showing time resolved images after addition of *H pylori*



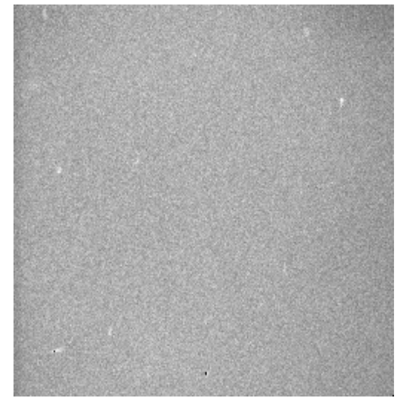
t = 0



t = 3 minutes



t = 27 minutes



t = 37 minutes

Increase in fluorescence background (~ pH), is directly correlated with the release of bacteria from confinement in the pores of the gel

In collaboration with Dr. Peter So, Biological Engineering, MIT.
Celli et al PNAS August 25, 2009 vol. 106 no. 34 14321-14326

Gastric Mucus: Home of *H. Pylori*

pH 2

Decreasing pH

pH 7

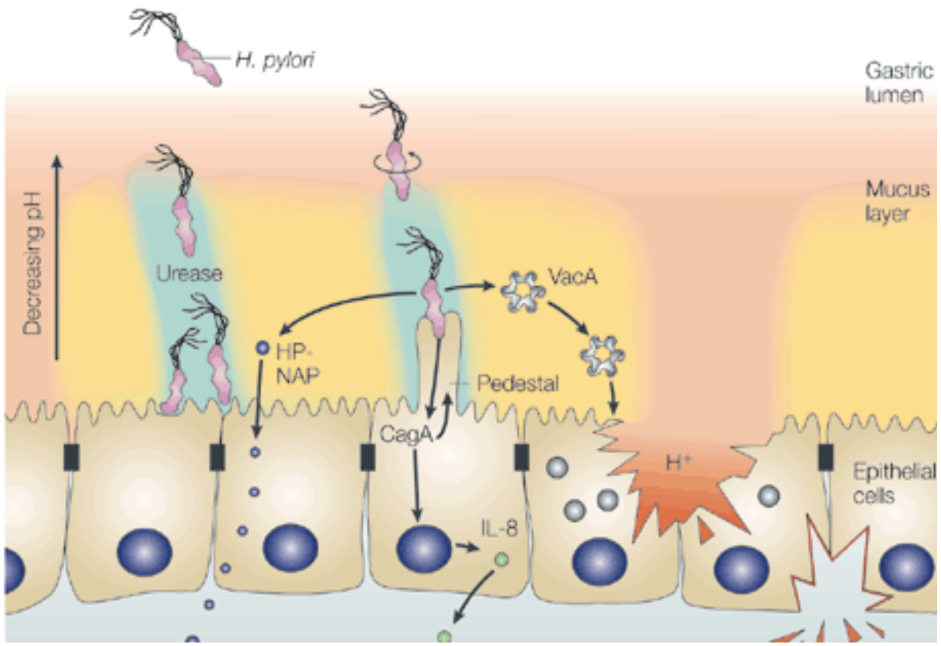
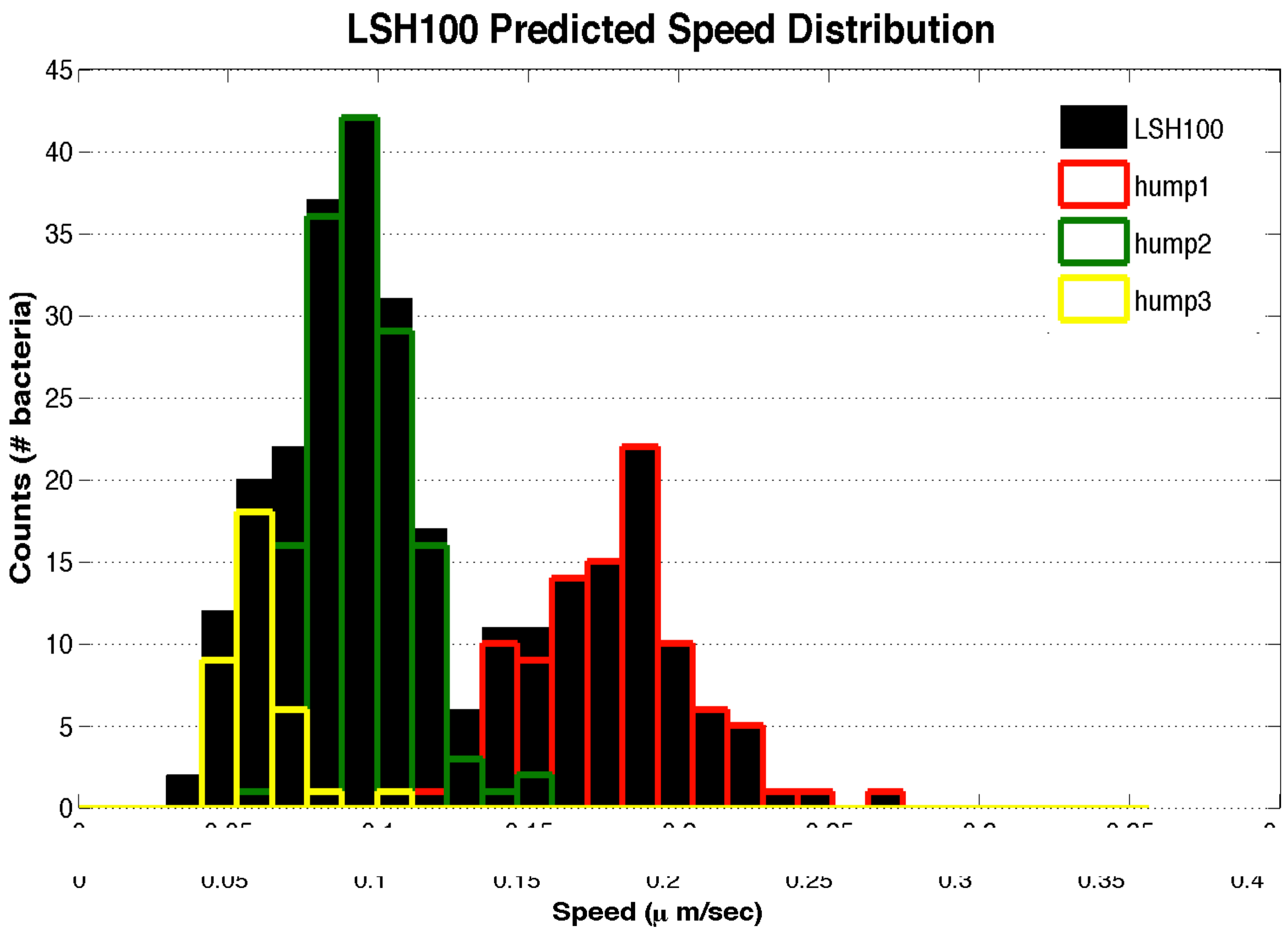


Image Credit: *C Montecucco and R Rappuoli* LIVING DANGEROUSLY: HOW *HELICOBACTER PYLORI* SURVIVES IN THE HUMAN STOMACH. NATURE REVIEWS: MOLECULAR CELL BIOLOGY **2** JUNE 2001, 457

Helicobacter Pylori (H. Pylori)
- Causes ulcers
- Spiral shaped
- Multiple flagella
- Swims through gastric mucus
- To survive at low pH it secretes urease to raise pH: $(NH_2)_2CO + H_2O \rightarrow CO_2 + 2NH_3$

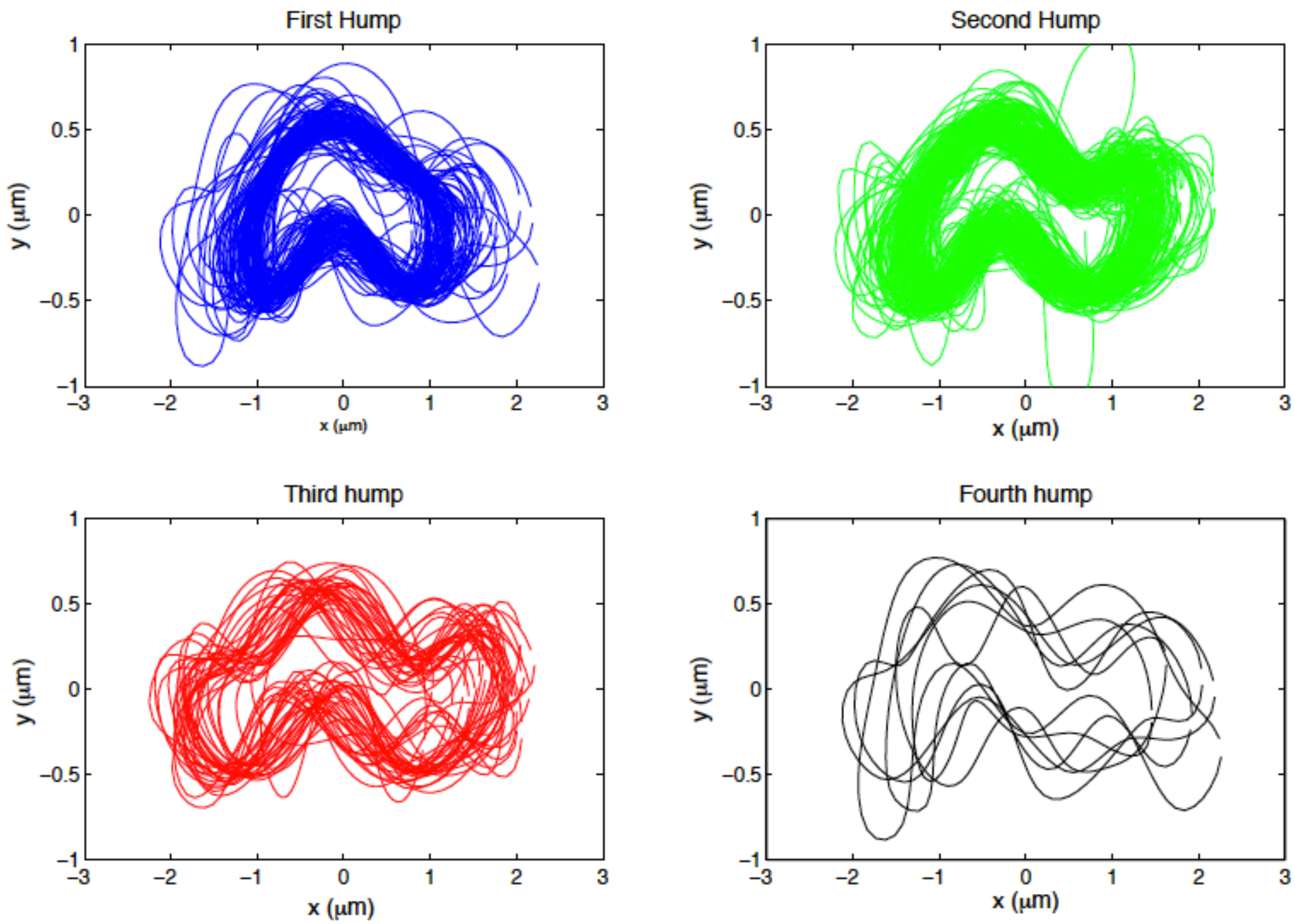
↑ ↓

Matrix (Gastric Mucus)
- Mucin → <u>viscoelasticity</u>
- Viscoelastic properties: η and $G^*(\omega) = G'(\omega) + iG''(\omega)$ are pH dependent



Speed ranging from 0 to 40 micron/sec

WT bacteria have different waveforms



Summary

HCl in stomach lowers pH. Gastric mucin forms gel.

→

H. pylori urease causes:
Urea → $NH_3 + CO_2$

HCl neutralized, pH ↑

→

Mucin transitions from gel to sol. Elasticity ↓

→

H. pylori swims freely through mucus and attaches to epithelial cells

Key Results:

- pH-Dependent rheological properties of gastric mucin: yield stress, shear thinning, effect of ionic strength variation, critical gel transition
- Bacteria swim in pH 7, but stuck in low pH
- Flagella rotation could be observed at pH 4, although there was no translation motion
- Bacterial motility parameters: Velocity, motor torque, spring constant

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  - Dr. Randy Ewoldt (MIT, Ph. D now at U. Minnesota)
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  - Nina Salama— Fred Hutchinson Cancer Center, Seattle, WA
  - Laura Martinez – FHC, graduate student

On a lighter note……

As the Frog in Disney's "The Princess and the Frog" says

"it's not slime—it's mucus"

Thank you!