

Can bacterial filaments regrow?

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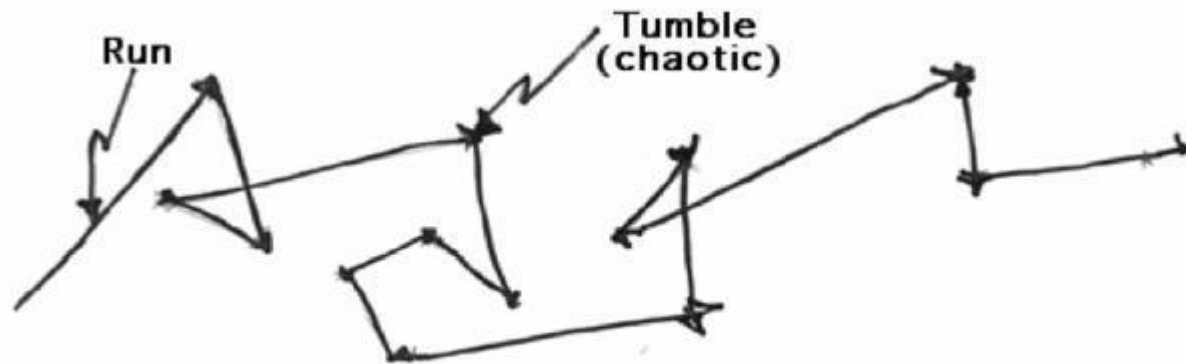


Namba Protonic NanoMachine Project
Osaka University, Japan.

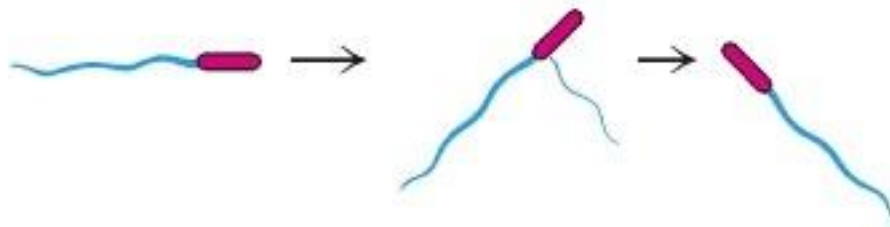
Why study bacterial motility?

- Toxicity and virulence increases with motility
- Antibiotics crisis
- Nanotechnology

3D biased random walk



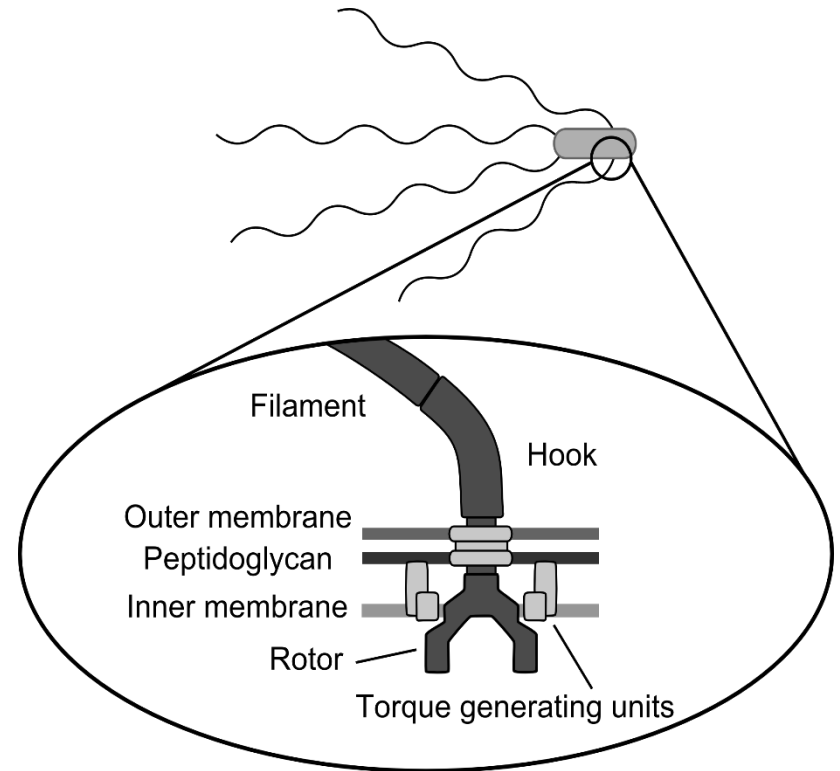
“Run-tumble” paradigm for taxis
Berg and Brown, Nature, (1972)



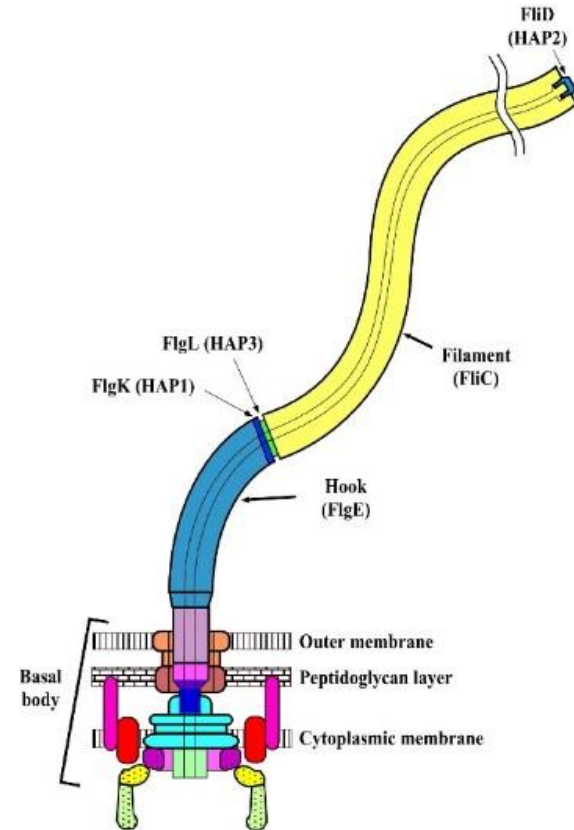
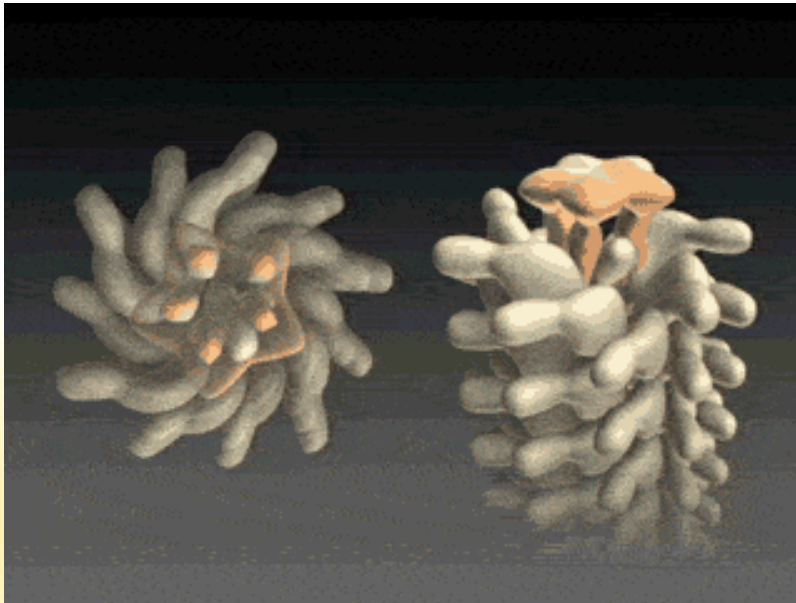
JM Berg, JL Tymoczko, and L Stryer. Biochemistry

How do bacteria move?

- Complex assembly of over 20 types of proteins
- 45nm-diameter rotative motor
- Rotation in both direction up 18000 rpm
- Proton powered



Filament structure

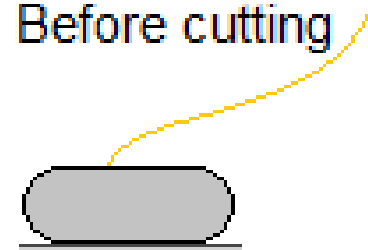


David E. Tanner and al. Theoretical and computational investigation of flagellin translocation and bacterial flagellum growth. *Biophysical Journal*

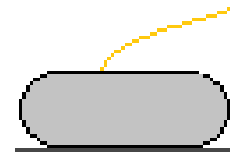
Methodology

- Cells are grown to the exponential phase of growth
- A first Fluorescent labelling is done
- Filaments are cut using a femtosecond laser
- Coordinates of the bacteria are noted
- A second labelling is done during a second growth period of 2h (37°C)
- Cells are revisited

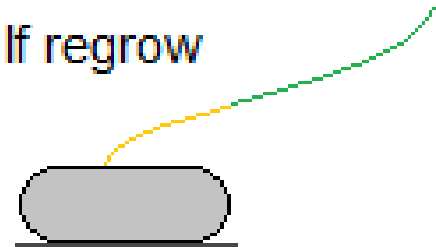
Before cutting



After cutting

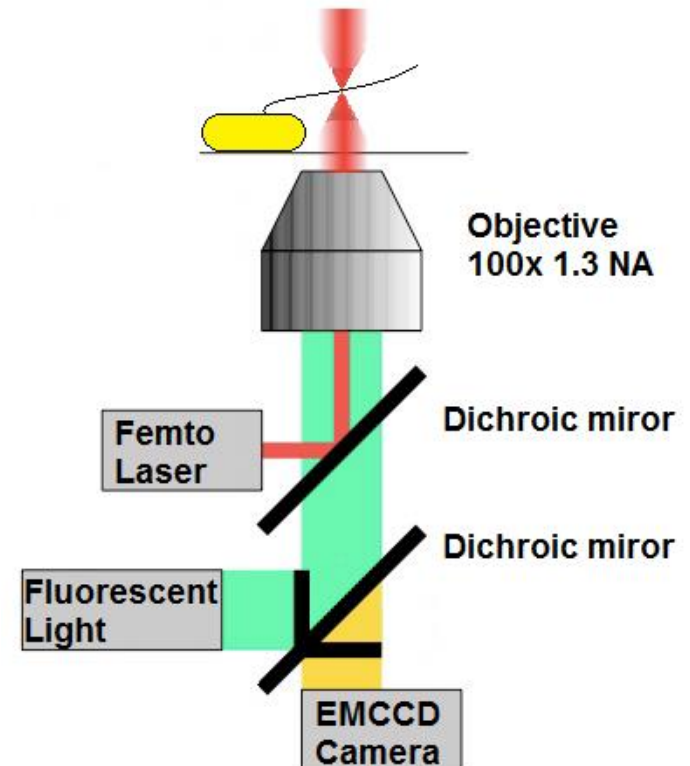
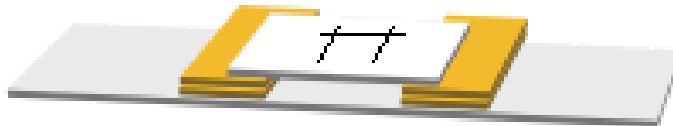


If regrow



Experimental setup

- Pulsed laser (1 khz)
80 fs, Near infrared
- Fluorescent microscopy
at 546nm and 488nm
- Custom-made flow-cell
- Bacteria with 1 or 2
filaments



Cutting the filament

- Laser is opened
- Filament cut itself on the laser beam
- Acceleration of the rotation shows cutting



Results after 2h growth period

- 44 filaments were cut and revisited

- No regrowth was observed

What we know

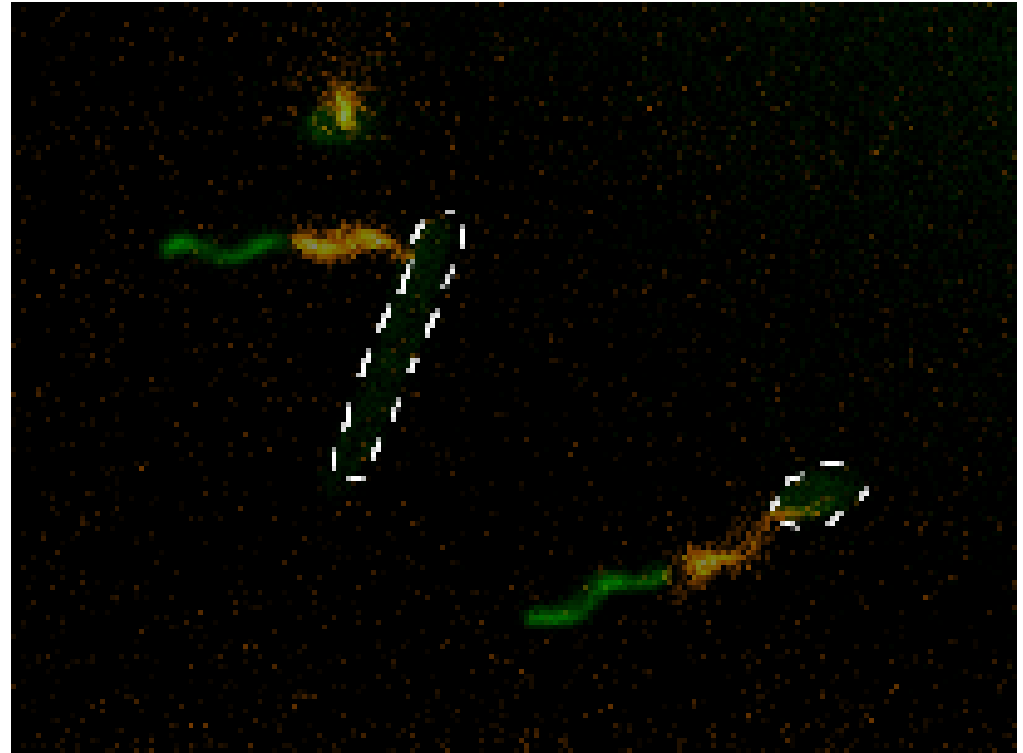
- When the filament is cut, the cap is lost
- The cap is needed for filament growth
- FliD protein is constantly secreted
- HAP3 (FlgL) is needed for the cap to form
- If there was regrowth, should take minutes to be seen

Overexpression of FliD (HAP2)

- A genetically modified strain controlling the expression of the FliD protein was used
- The protein was overexpressed during the 2h growth period
- 18 filaments were cut and revisited
- No regrowth was observed

Controls

- Bacteria in the same flow-cell left untouched were used as controls
- 90-95% of filaments still turning were bicolor



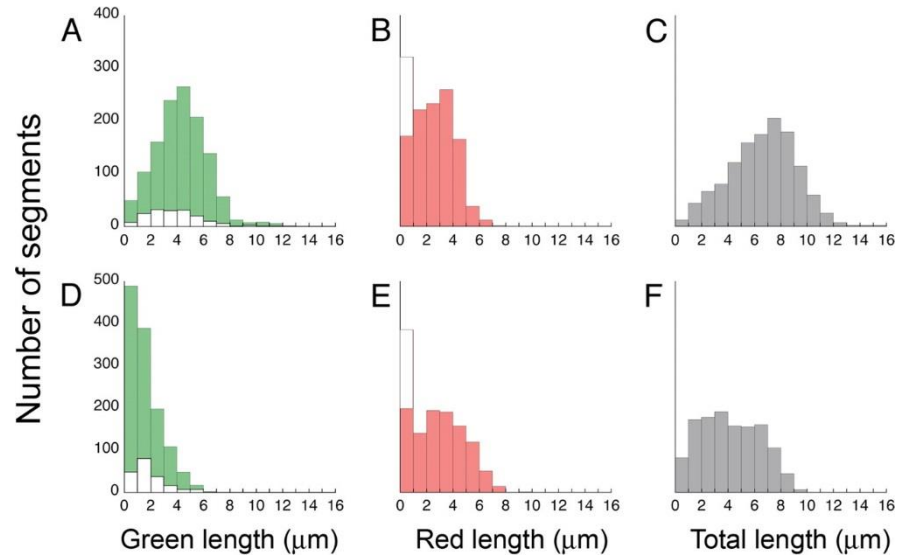
Controls (2)

- In one instance, a second filament had grown on a cell with a cut filament



Previous study

- Turner and Berg (2012)
 - Cells were sheared mechanically
 - Let overnight at 7°C
 - Tends to show regrowth



Turner L et al. *J. Bacteriol.* 2012;194:2437-2442

Conclusion

- No regrowth was observed
- Bicolor uncut filaments in the same flowcells were observed
- Even with higher levels of cap protein, cut filaments don't regrow
- In these conditions, bacterial filaments don't regrow

Thank you



Fonds de recherche
sur la nature
et les technologies



- Dr Simon Rainville
- PhD students
 - Guillaume Paradis
 - Ismael Duchesne
- Dr Kelly Hugues
University of Utah
- Dr Marc Erhardt
Helmholtz Centre for
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