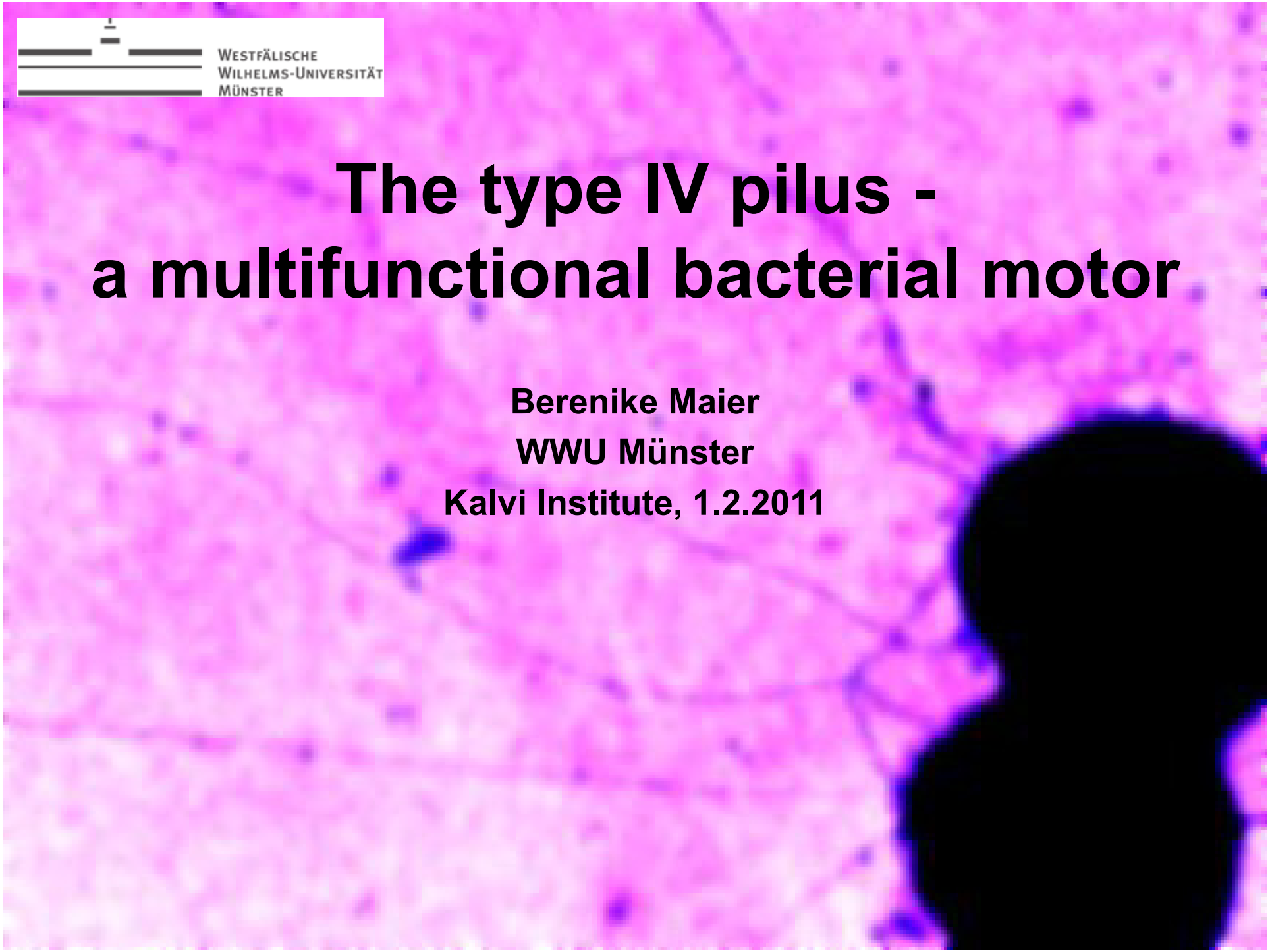
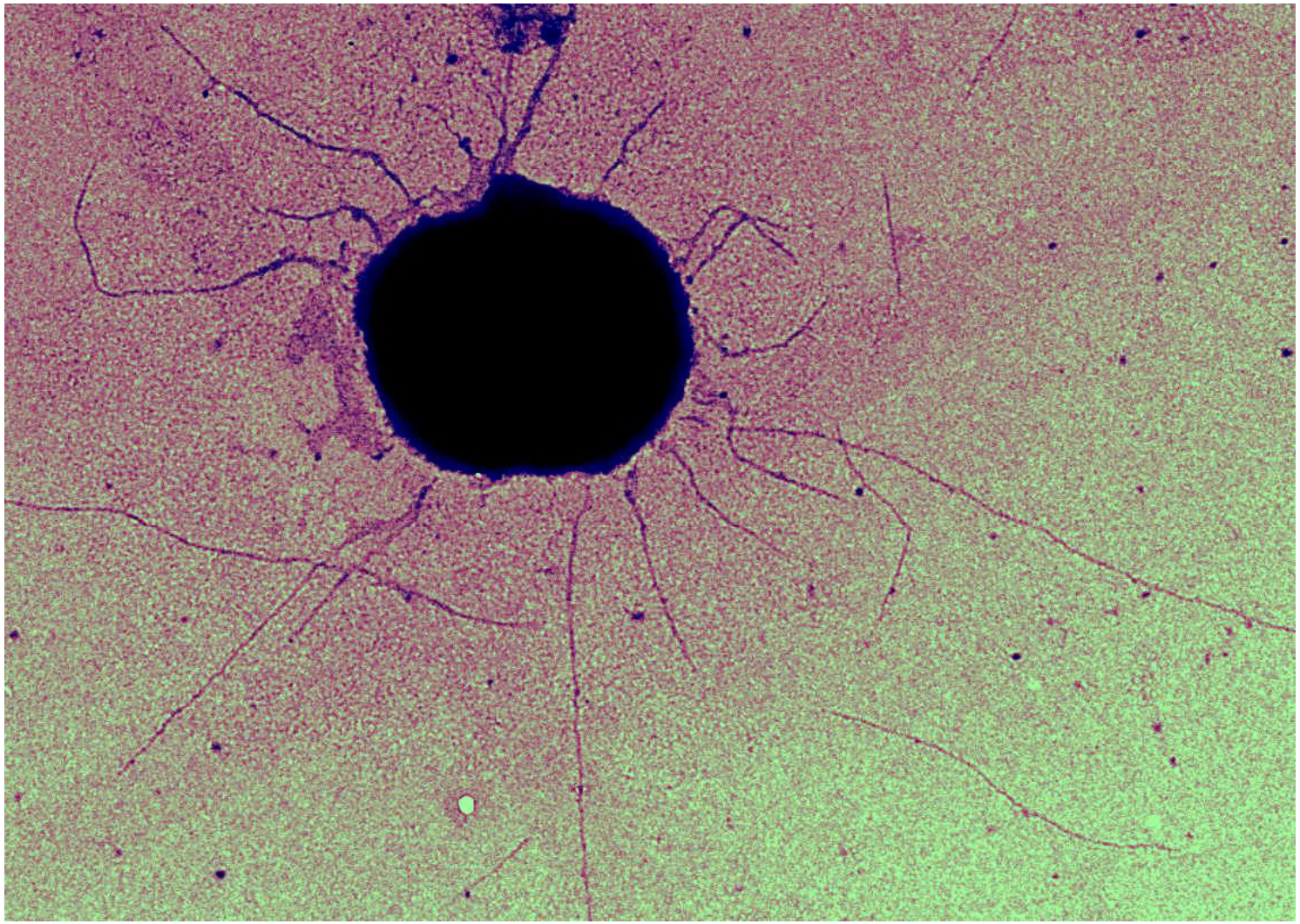


Westfälische Wilhelms-Universität Münster

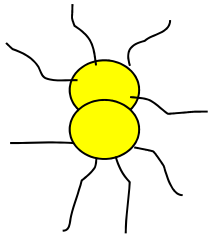
The type IV pilus - a multifunctional bacterial motor

Berenike Maier
WWU Münster
Kalvi Institute, 1.2.2011

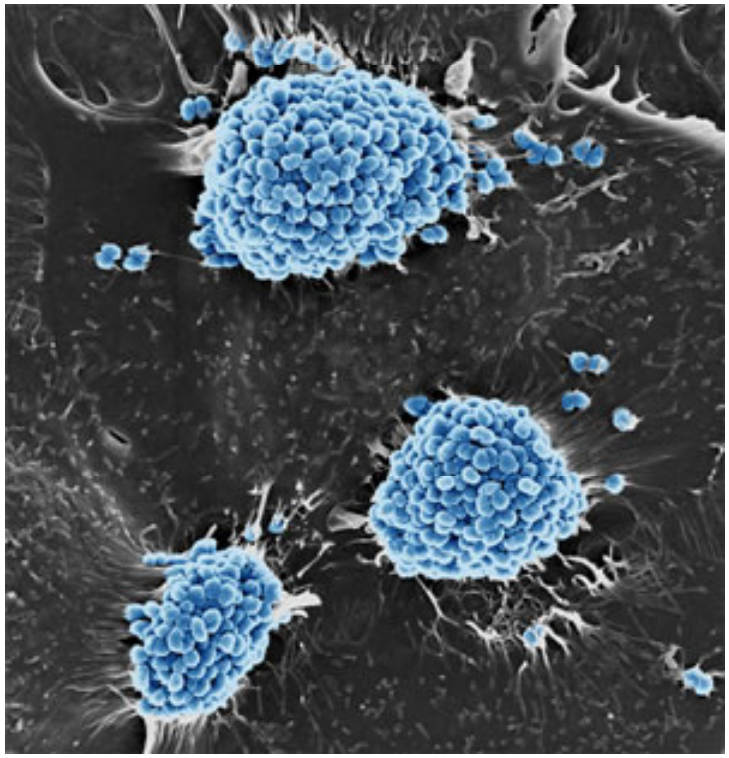
Type IV pili are ubiquitous multifunctional polymers



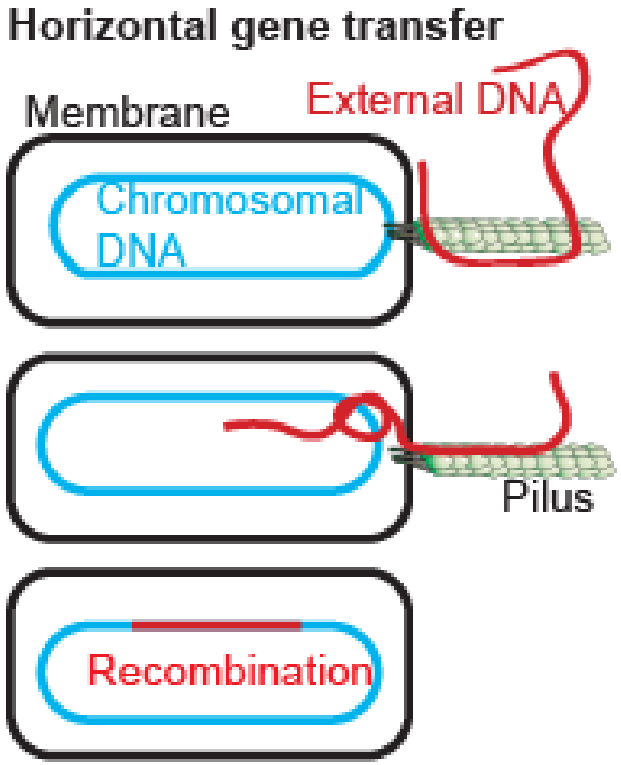
Type IV pili are ubiquitous multifunctional polymers



Neisseria gonorrhoeae:
genome size 2Mb



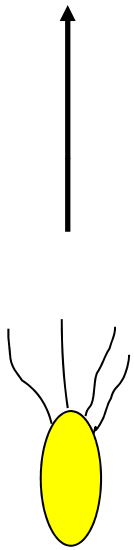
Higashi et al, 2007

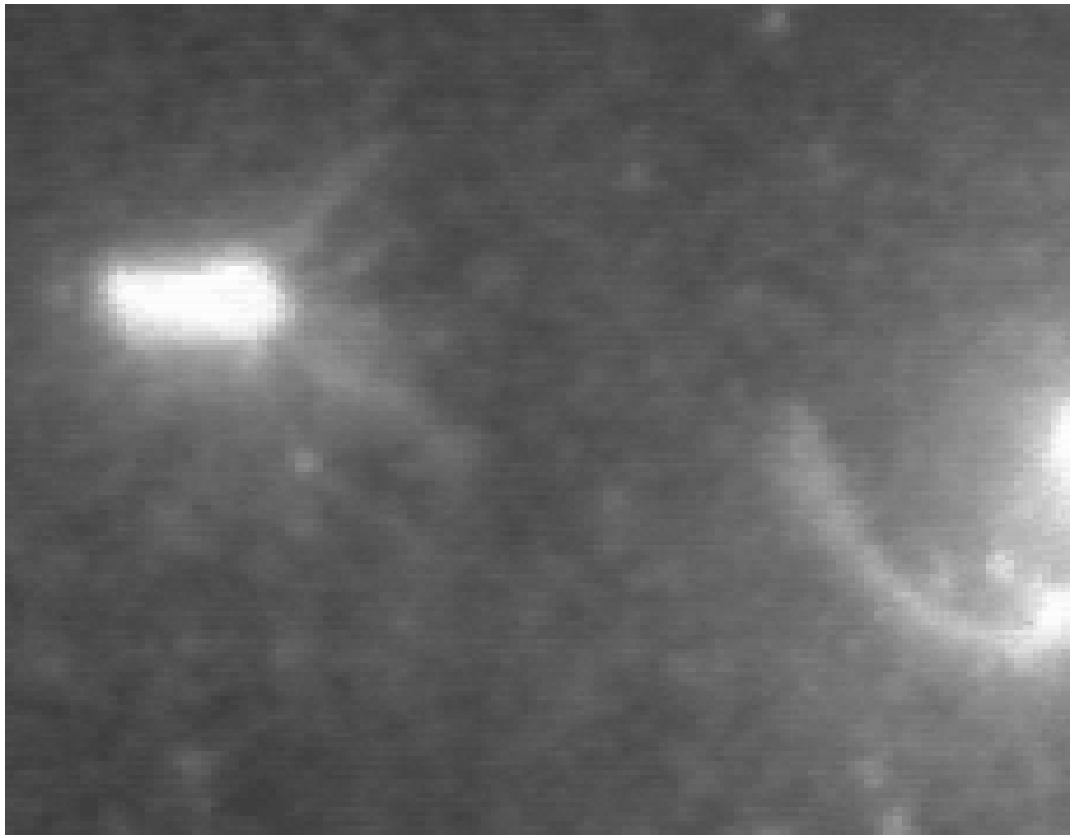


Functions include:
virulence, adhesion, microcolony formation, biofilm formation, motility, gene transfer

Type IV pili are ubiquitous multifunctional polymers

Pseudomonas aeruginosa:





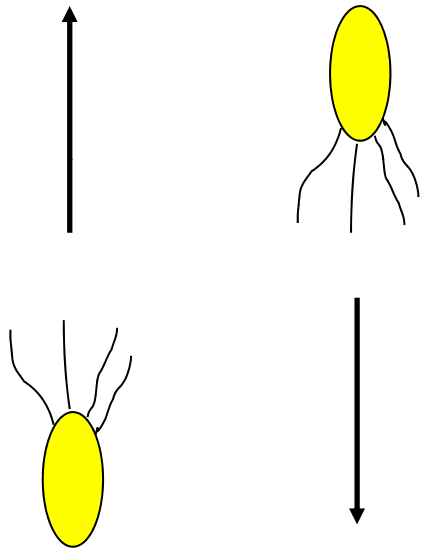
Skerker, Turner, Berg, PNAS 2001

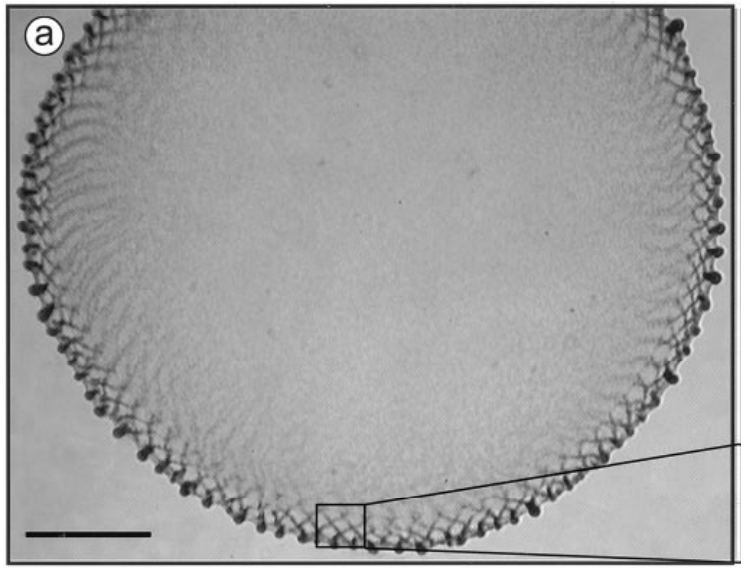
Functions include:
adhesion, motility, biofilm formation, lipid-based chemotaxis

Type IV pili are ubiquitous multifunctional polymers

Myxococcus xanthus:
genome size 9Mb

8 min reversal time



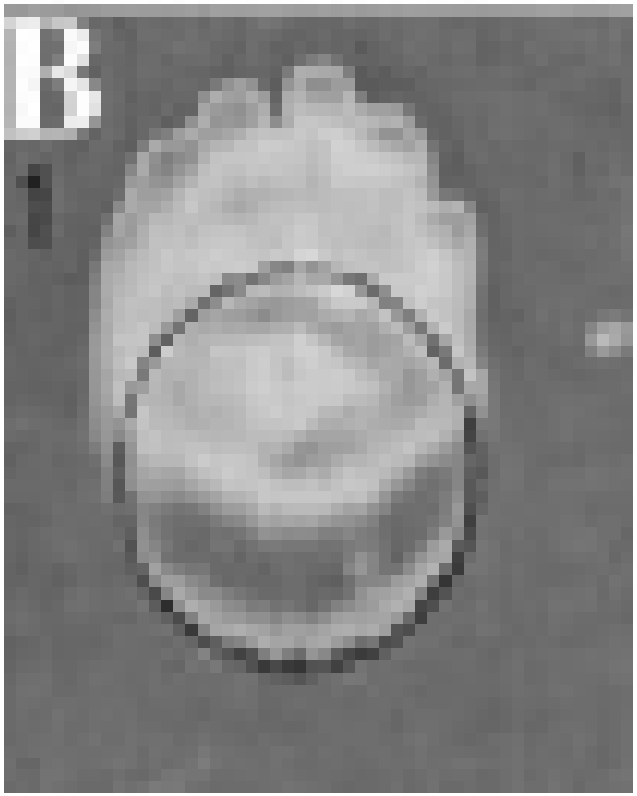


Welch & Kaiser, PNAS 2001

Functions include:
adhesion, motility, fruiting body formation, lipid-based chemotaxis

Type IV pili are ubiquitous multifunctional polymers

Synechocystis PCC6803*:*

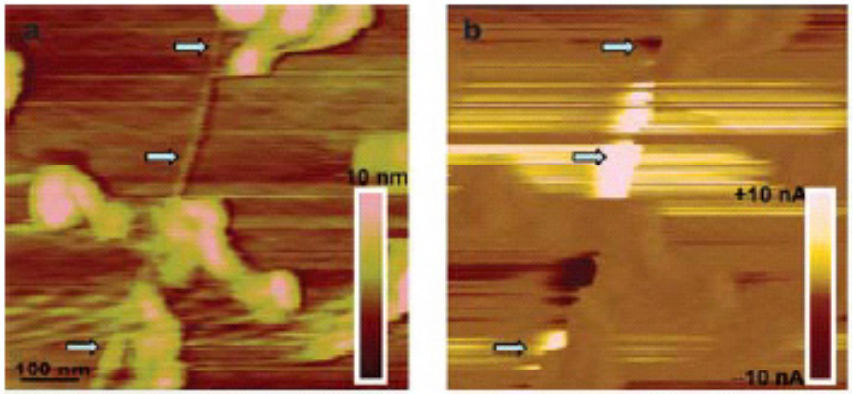


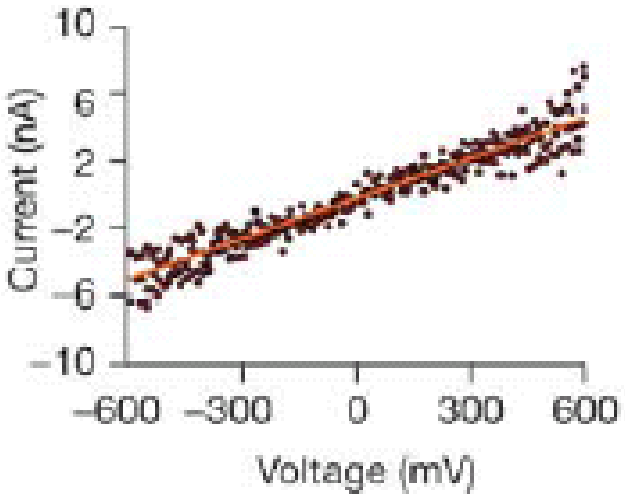
Bhaya et al PNAS 2002

Functions include:
motility, phototaxis

Type IV pili are ubiquitous multifunctional polymers

Geobacter sulfurreductens:





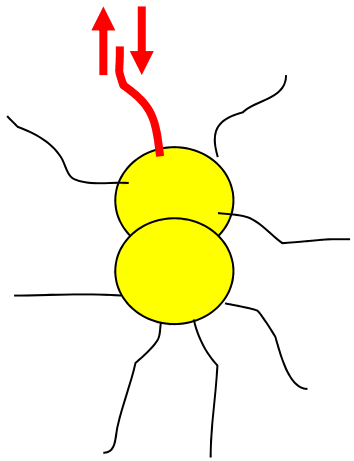
Functions include:
electron transfer

Reguera et al. Nature 2005

The type IV pilus is ubiquitous amongst phylogenetically diverse bacterial species and support different functions

Question: Have the biophysical properties of pili adapted differently to support different phenotypes?

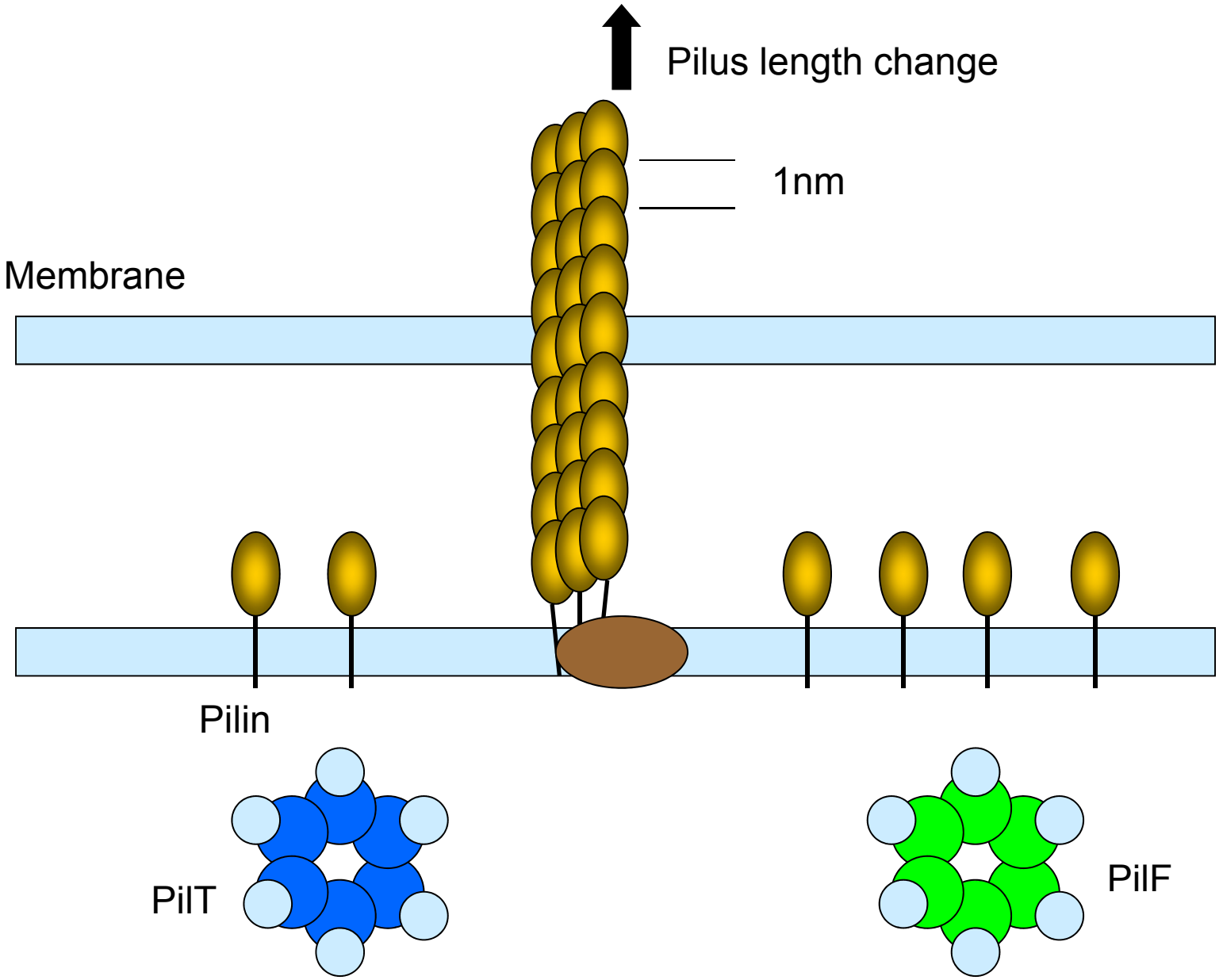
Biophysics of individual pili



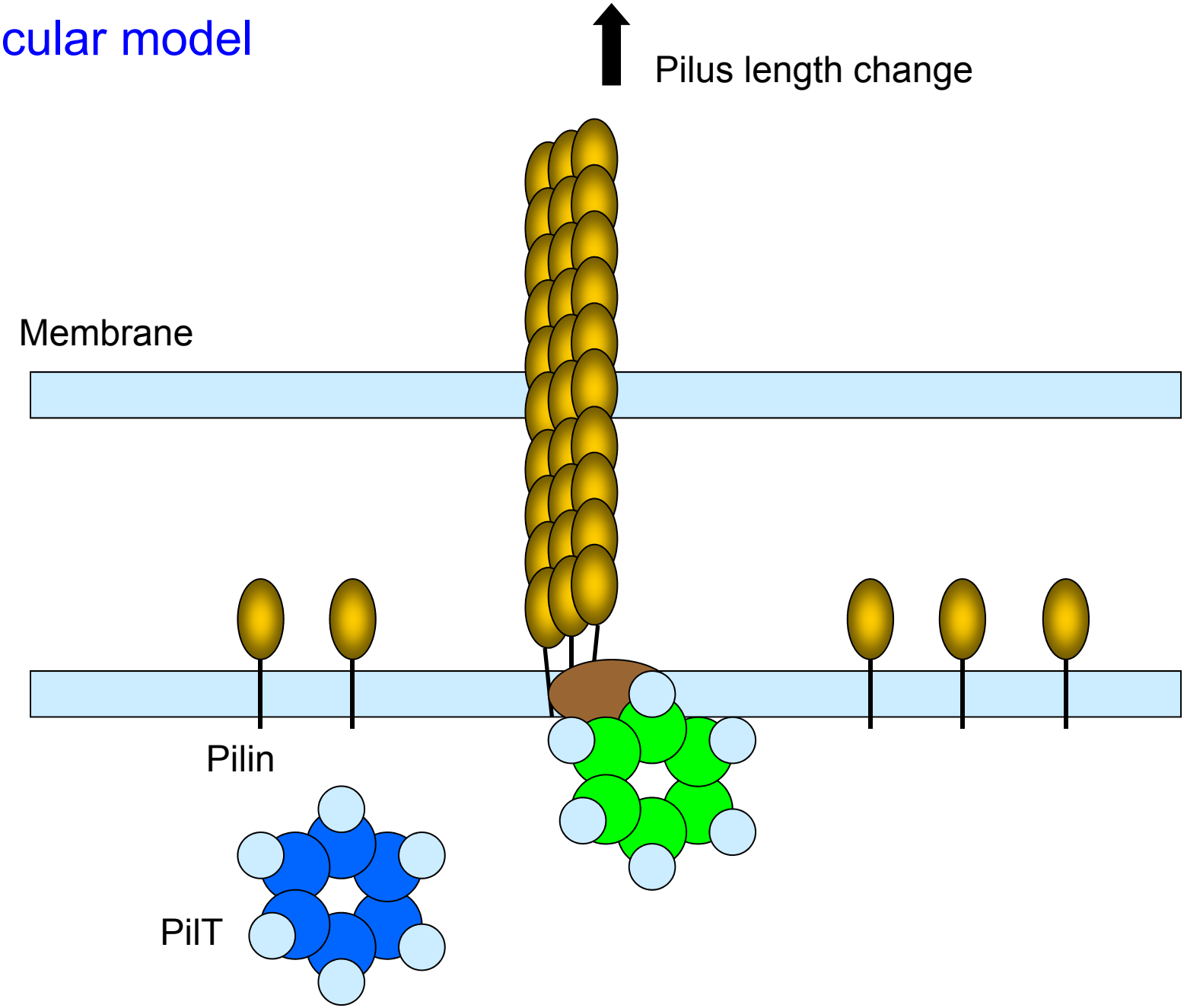
Martin Clausen, Rainer Kurre

Michael Koomey, Oslo University

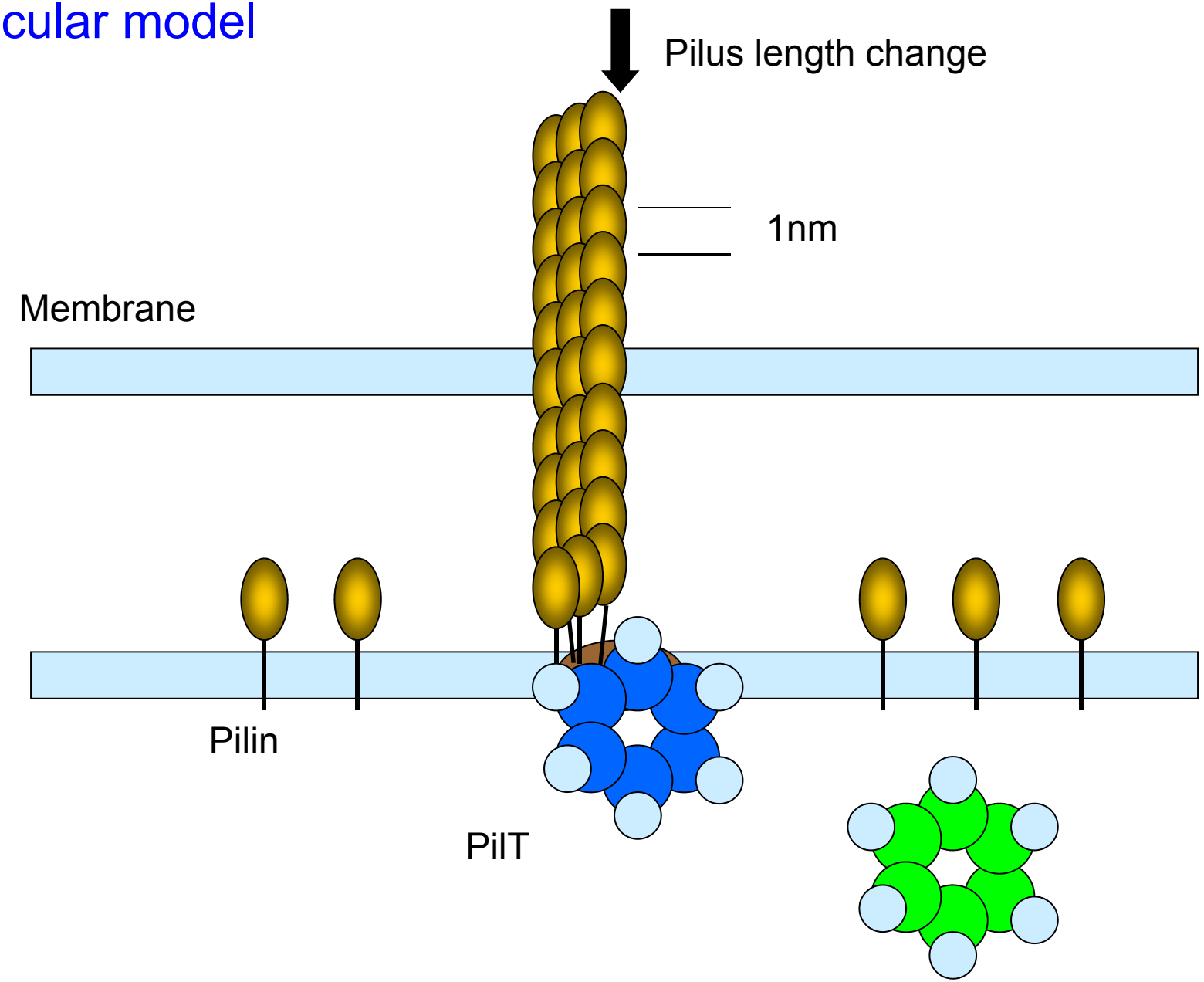
Molecular model of pilus dynamics



Molecular model



Molecular model



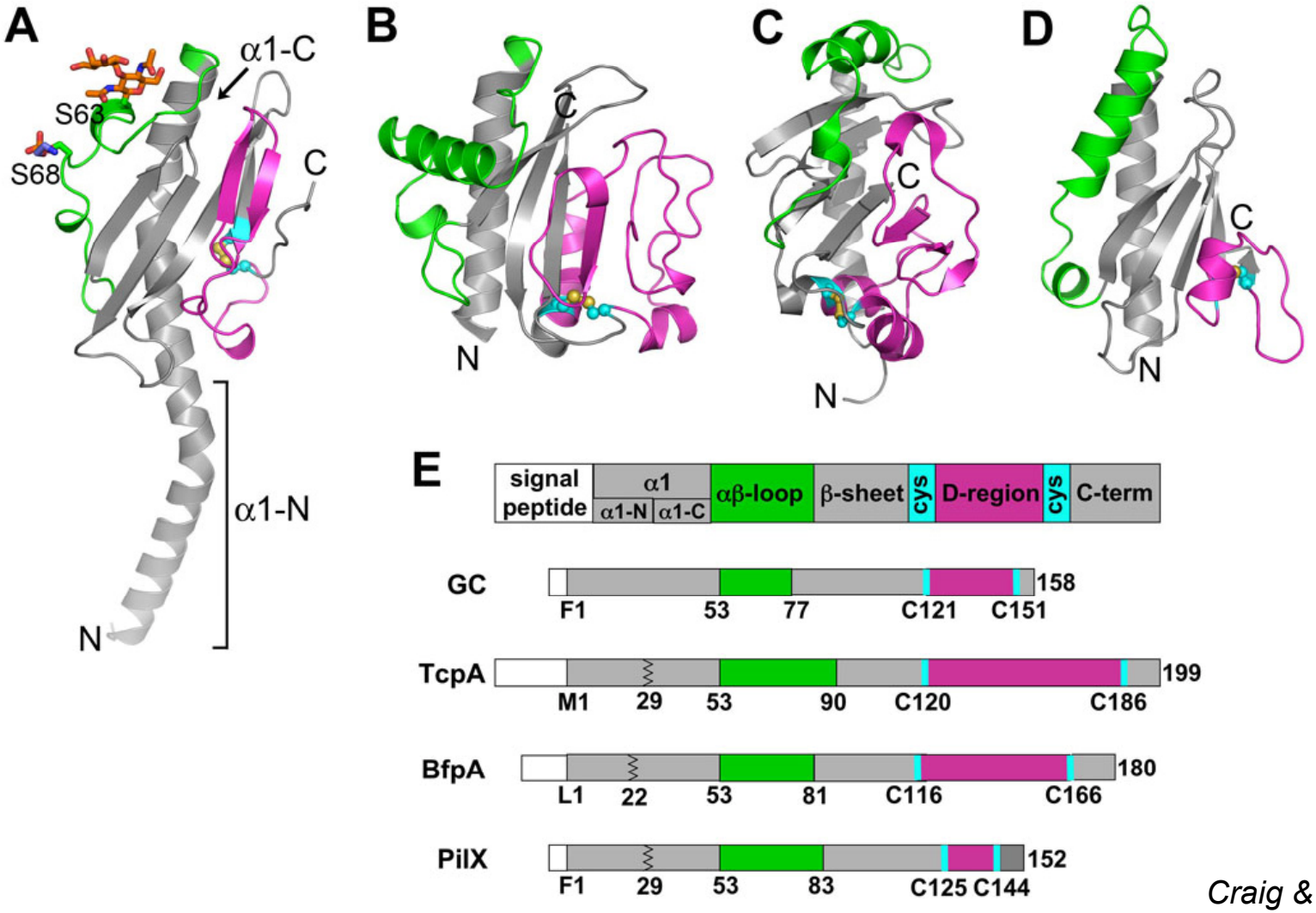
Pilin structure of different species

Neisseria gonorrhoeae

Vibrio cholerae

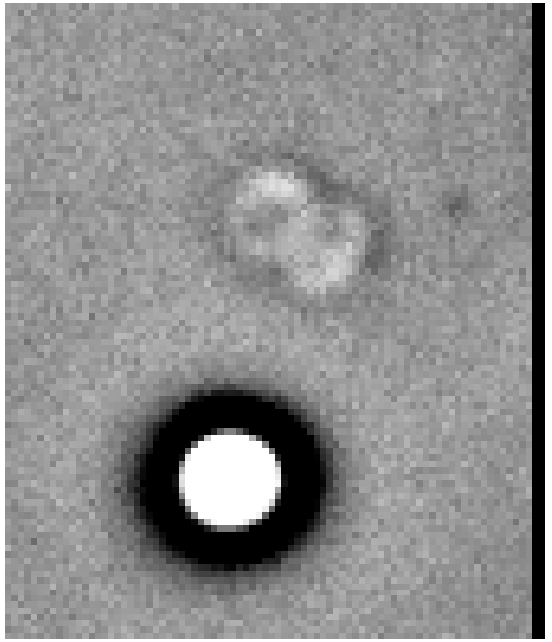
enterophathogenic *Escherichia coli*

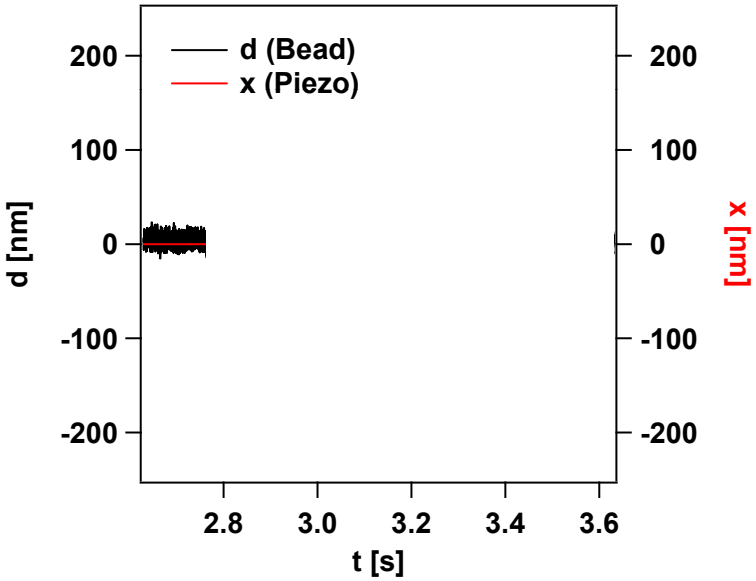
minor pilin *Neisseria menigiditis*

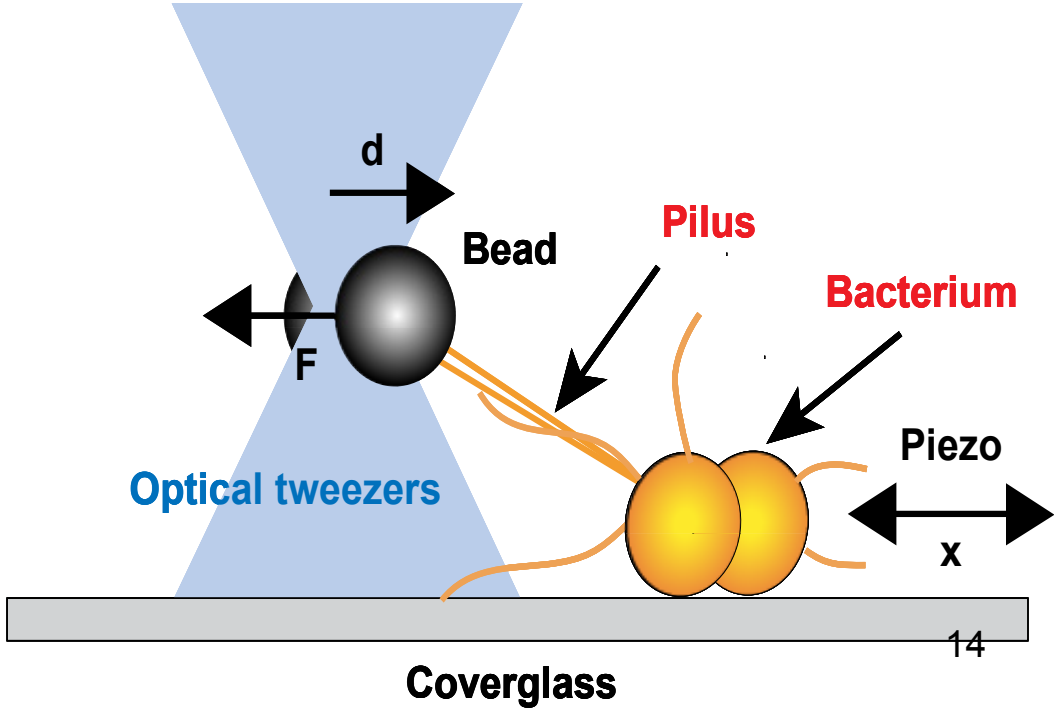


Craig & Li, 2008

Single pilus dynamics measured with clamped force

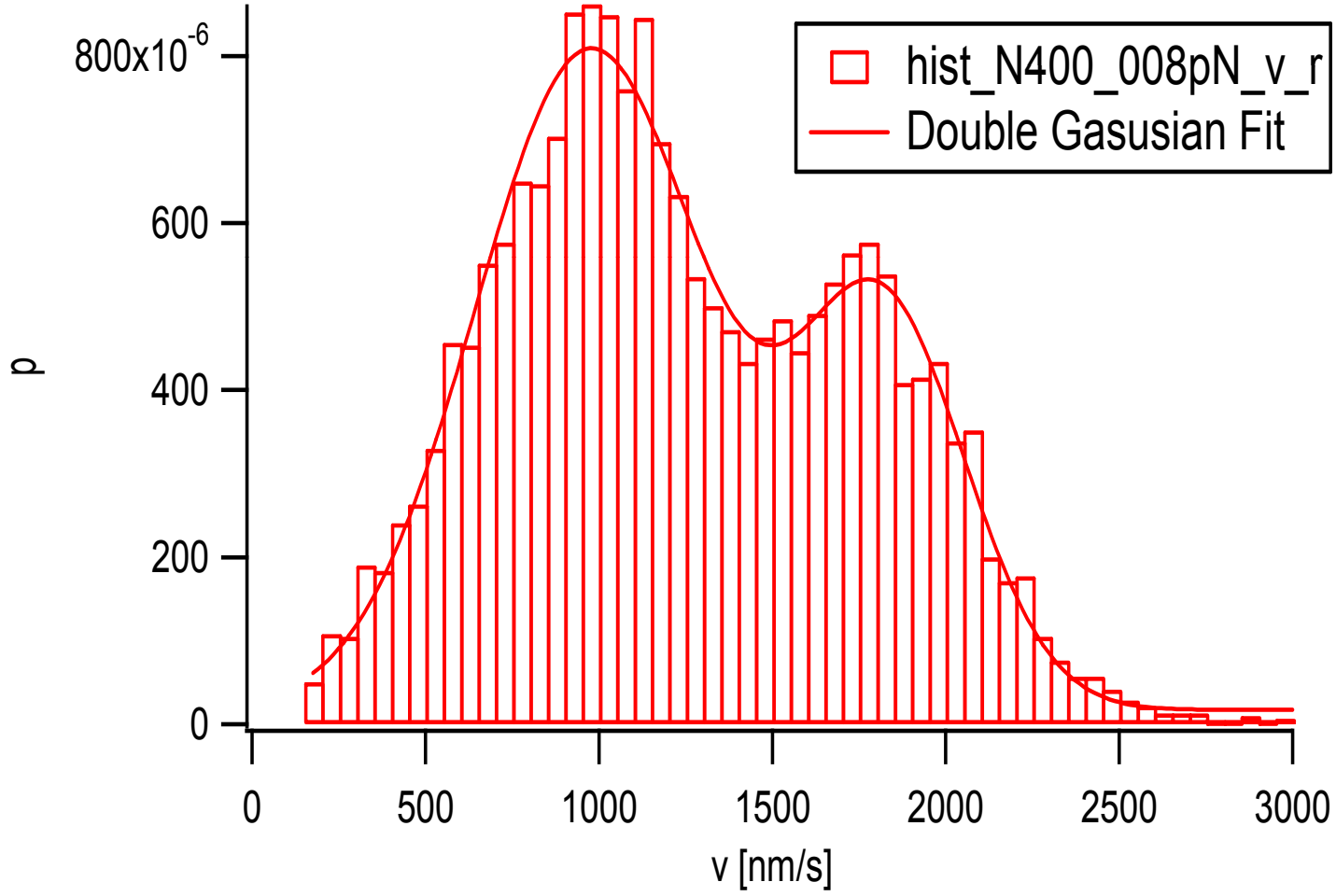






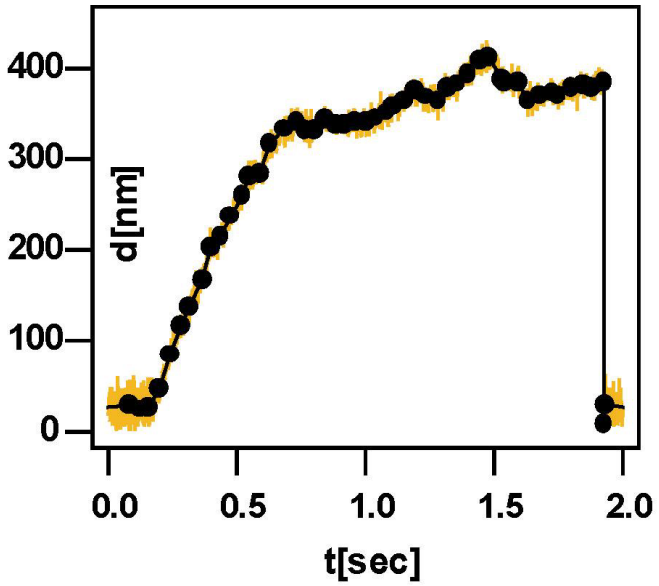
Velocity of type IV pilus retraction is bimodal

N. gonorrhoeae

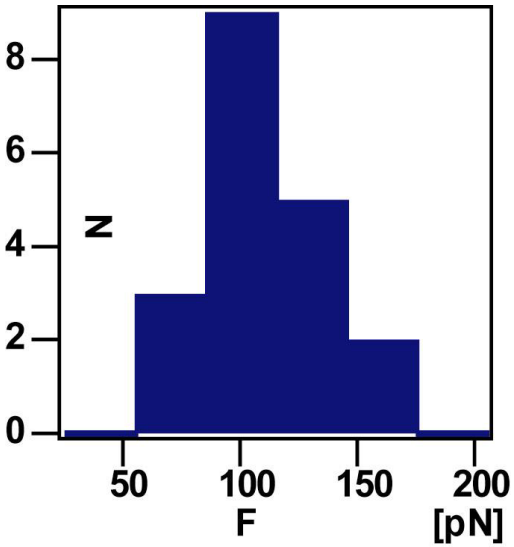


Type IV pili generate force > 100pN by retraction

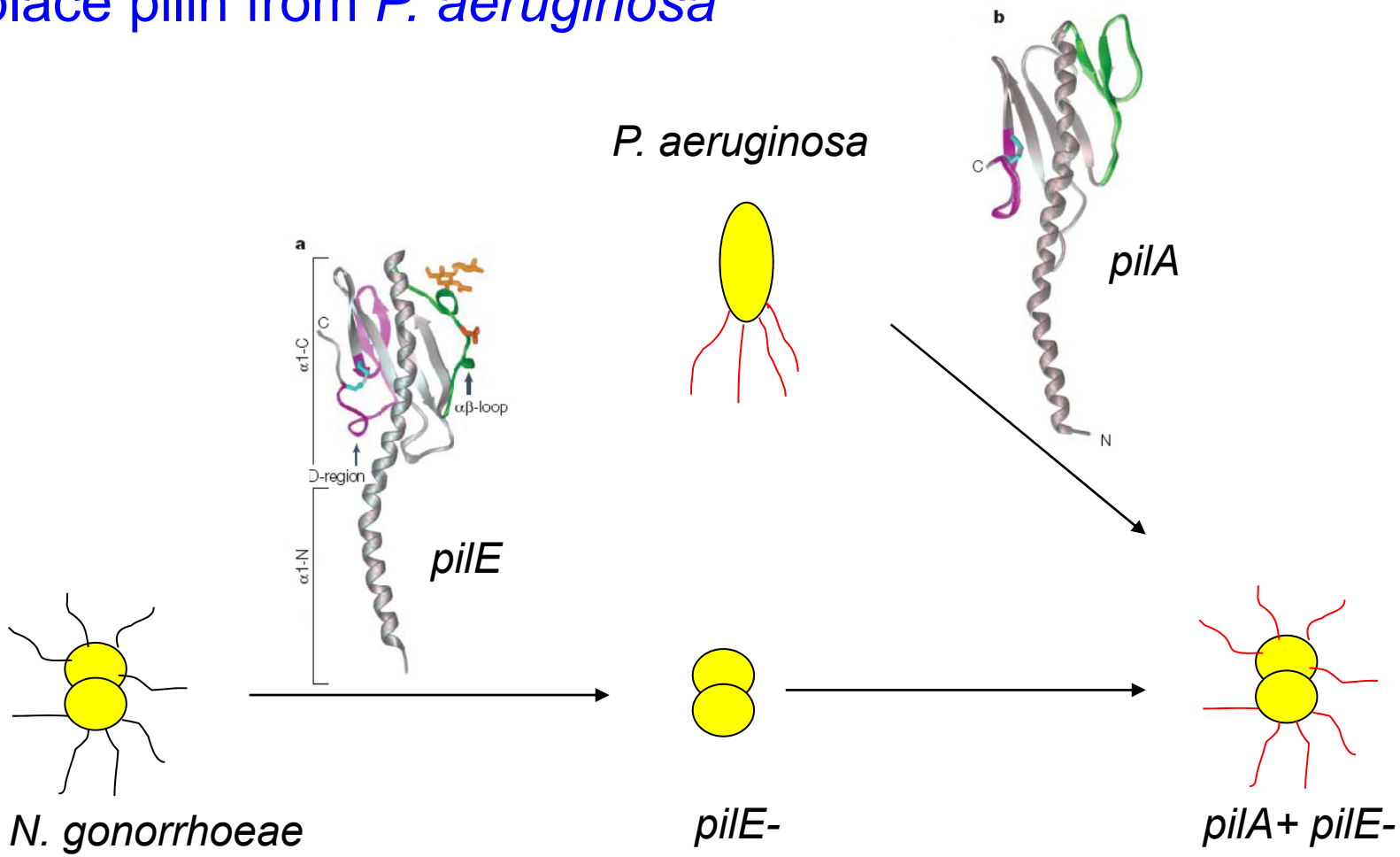
Displacement of the bead from the center of the laser trap:



Distribution of stalling forces:



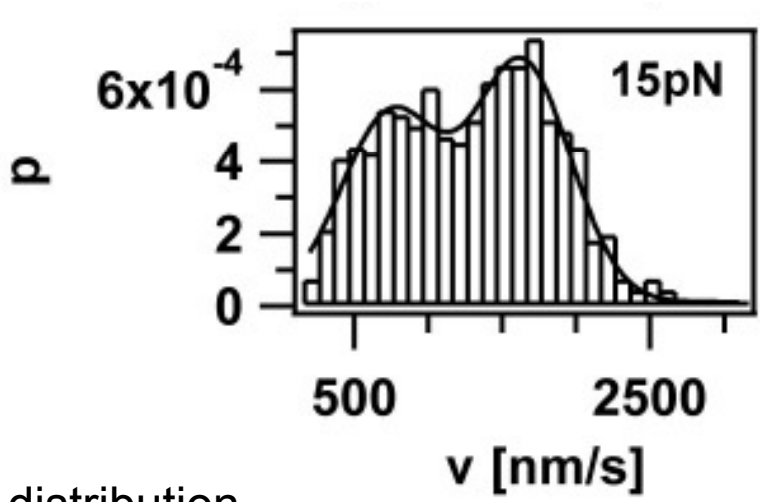
Replace pilin from *P. aeruginosa*



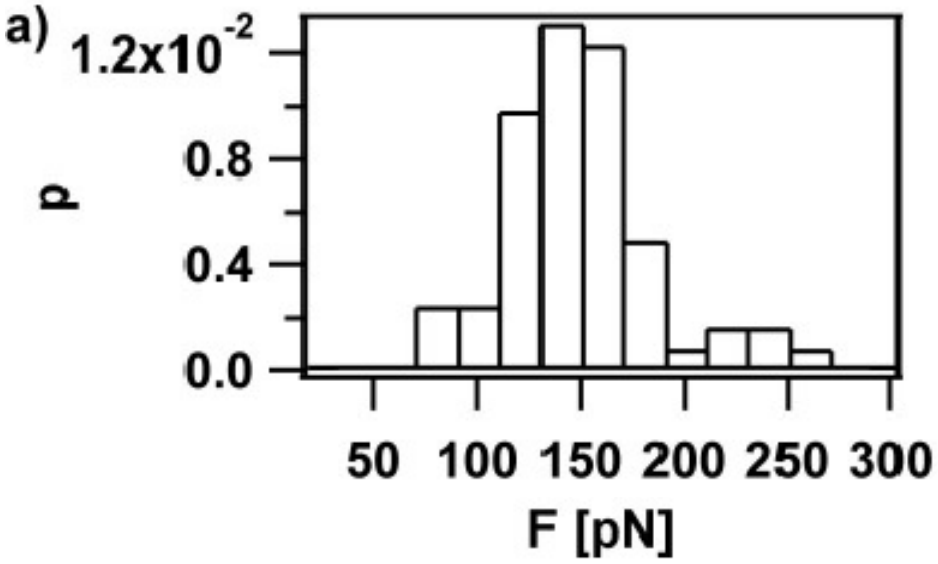
Result: comparable velocity and stalling force

Velocity and force generation in *M. xanthus*

Velocity distribution



Force distribution



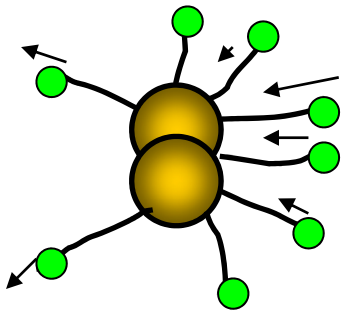
Conclusion

The biophysical properties of T4 pili are well conserved at the level of a single pilus.

What about velocity regulation?

Maier et al, PNAS 2002
Winther-Larsen et al J. Bacteriol. 2007
Clausen et al, J. Bacteriol 2009
Clausen et al, Biophys. J. 2009

How is pilus dynamics translated into movement?

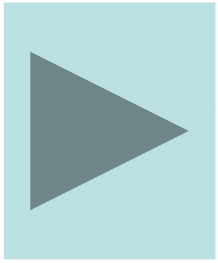


Claudia Holz, Dirk Opitz, Rainer Kurre, Andrea Höne

Lilo Greune, Alexander Schmidt, ZMBE Münster

Iryna Bulhya, Lotte Sogaard-Andersen, MPI Marburg

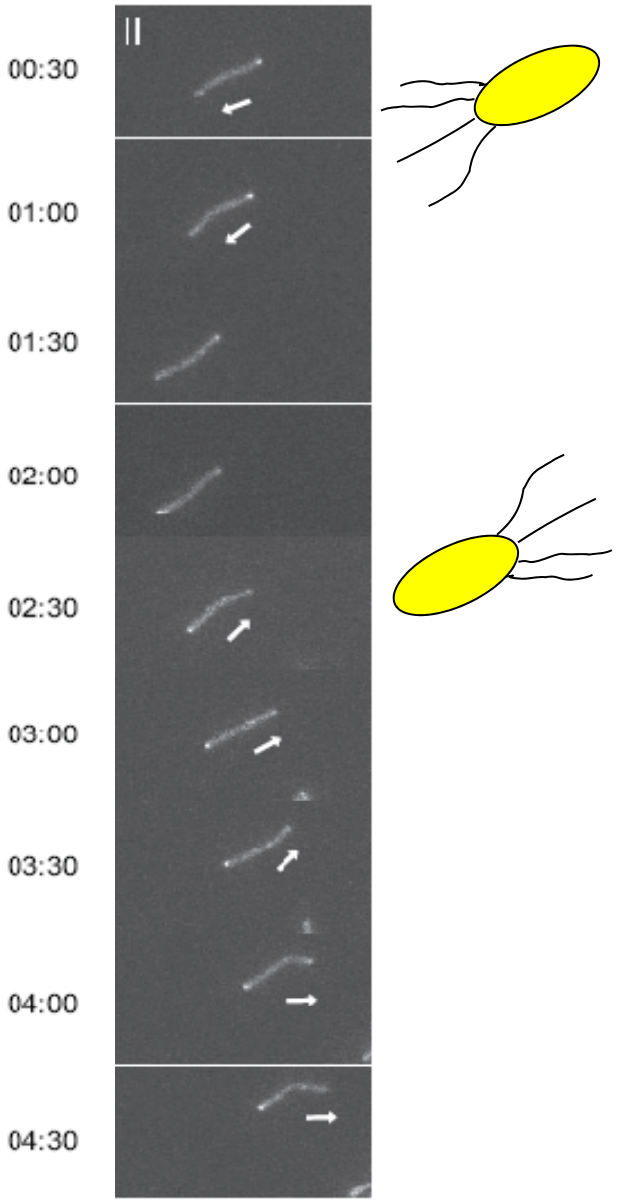
M. xanthus: Coordinated movement

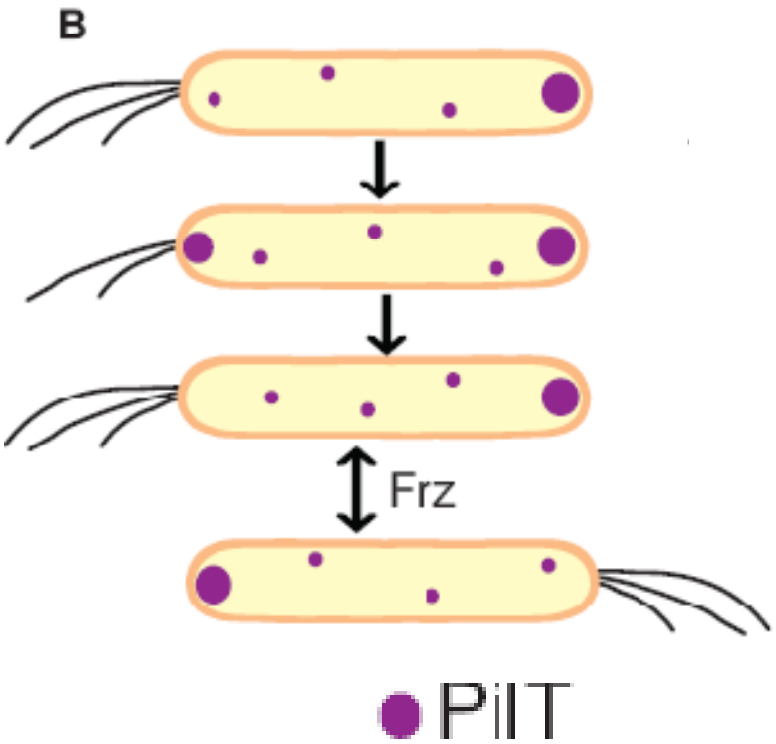


Sliusarenko et al, PNAS 2006

M. xanthus: PilT foci are well coordinated during directional reversal

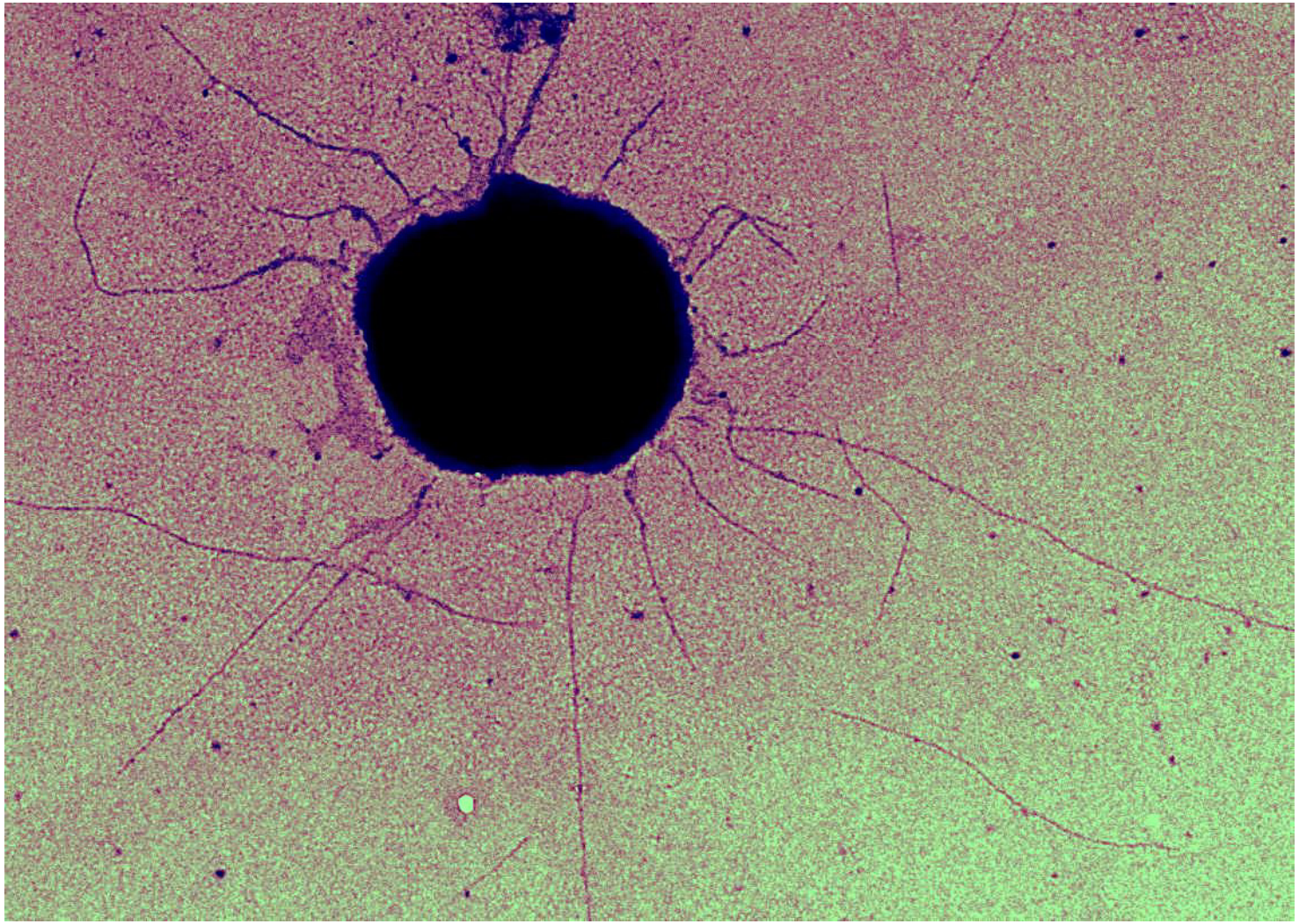
ΔpilT/yfp-pilT:





Pili are randomly distributed around *N. gonorrhoeae*

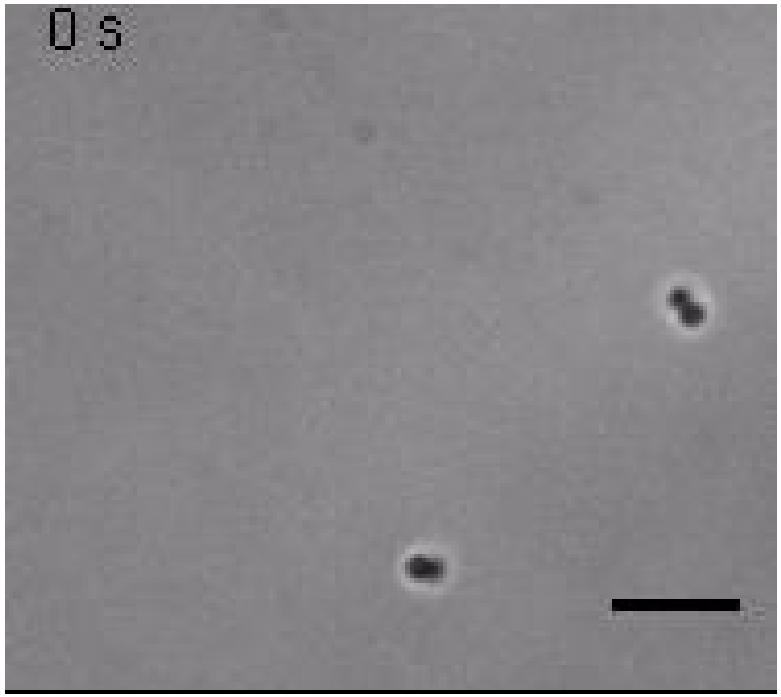
TEM of motile bacterium:

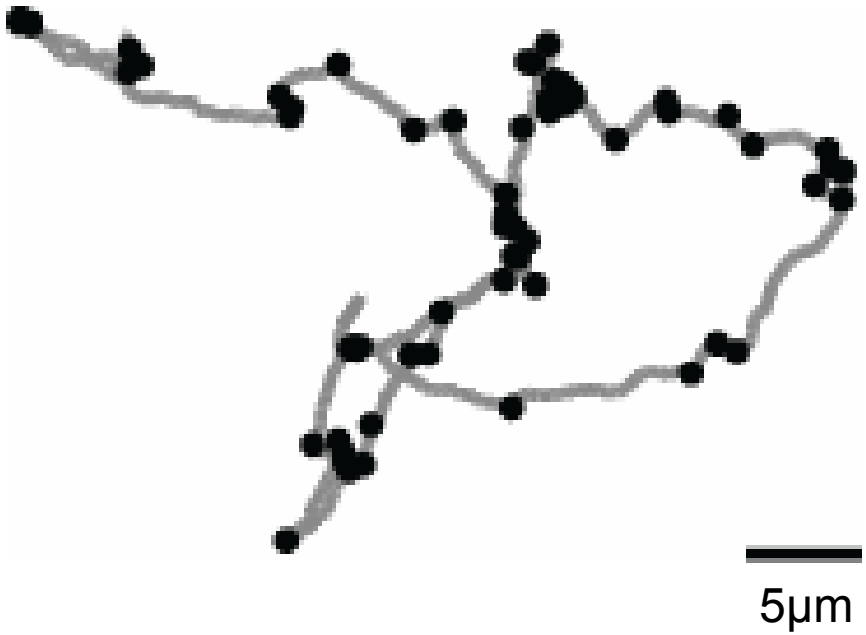


Twitching observed with 3-27 pili

Bacterial movement is persistent

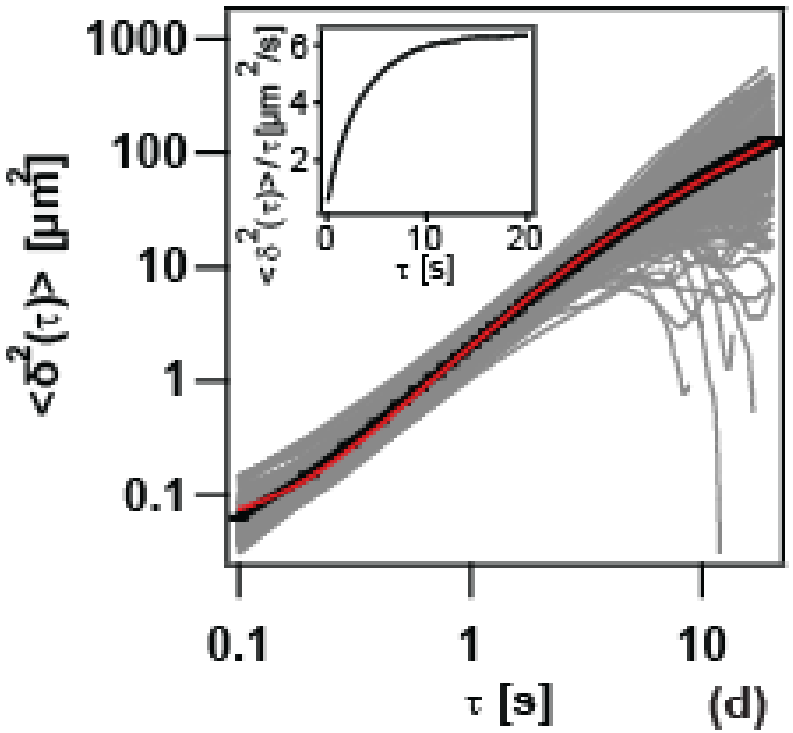
Typical movement of *N. gonorrhoeae* on BSA-treated glass





Bacterial movement is persistent

Mean squared displacement ($<\delta^2(\tau)>$) of bacteria:

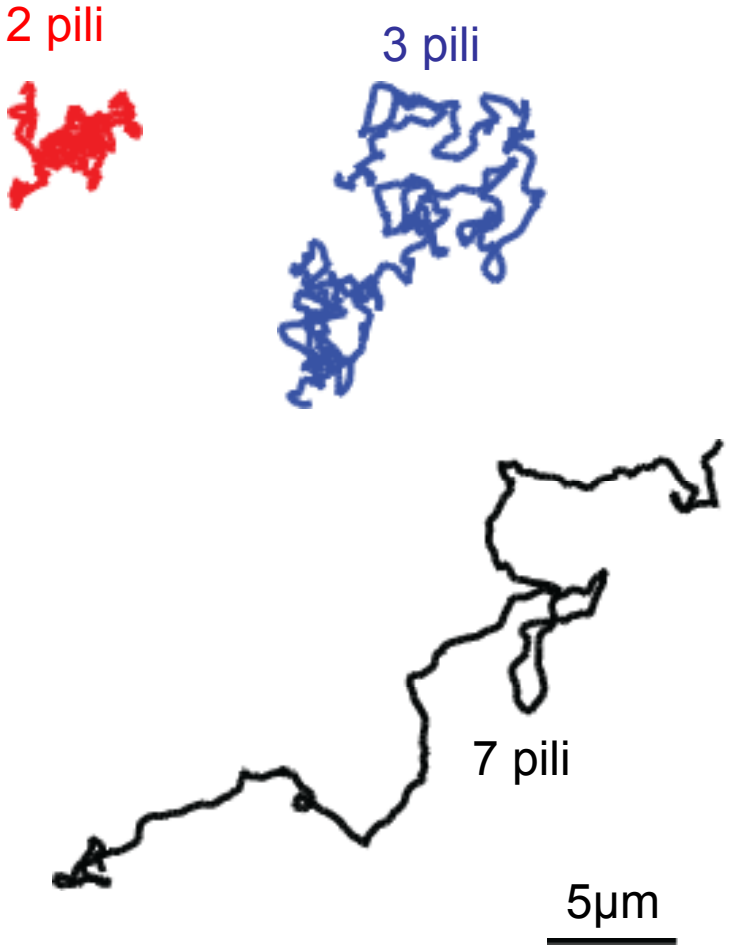


$$\left\langle \delta^2(\tau) \right\rangle = 2\tau_c v^2 \left(\tau - \tau_c \left(1 - e^{-\frac{\tau}{\tau_c}} \right) \right) + A$$

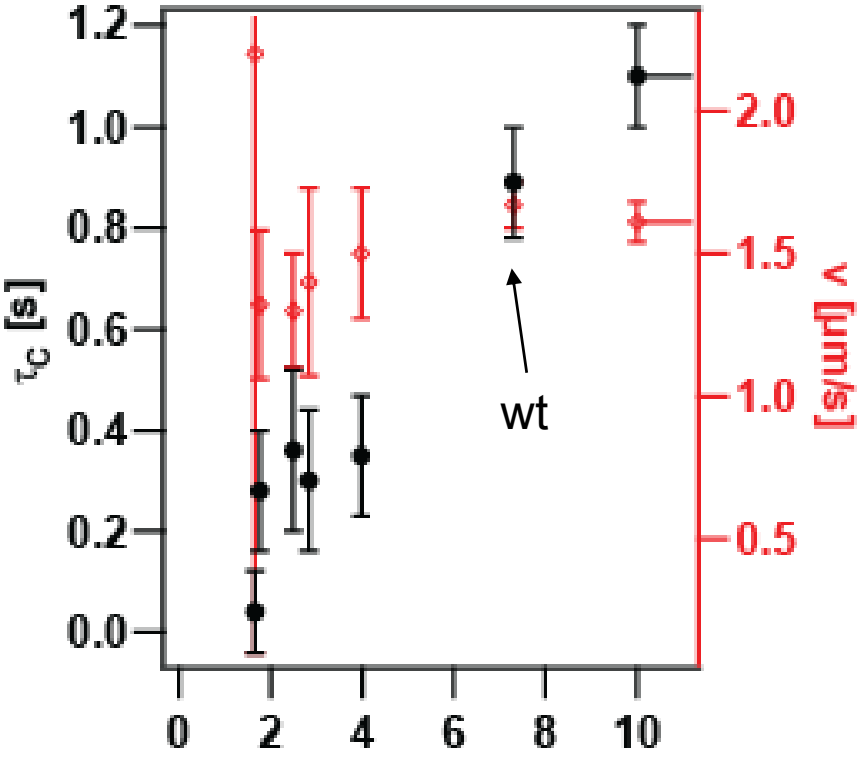
τ_c = (1.4 ± 0.2) s
v = (1.6 ± 0.1) µms^{-1}

=> correlation time τ_c longer than pilus retraction time

Multiple pili are required for persistent movement

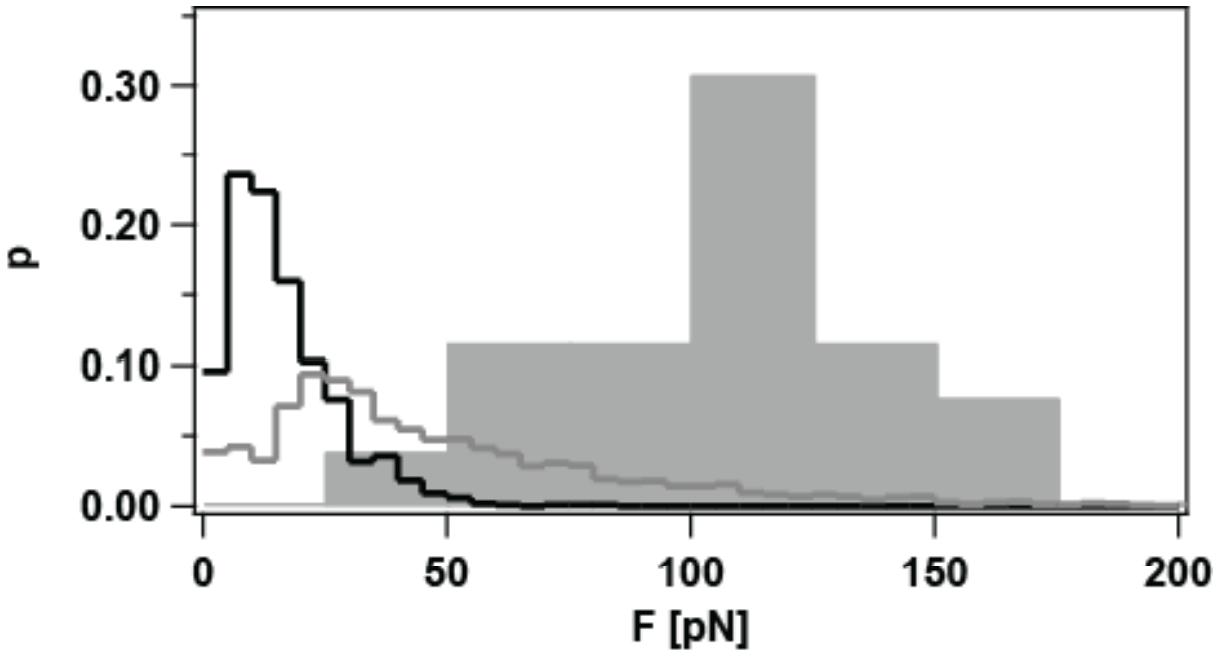


Correlation time τ_c:
Velocity *v*:



The unbinding force is significantly lower than the motor force

Distribution of forces *F*:



Unbinding force from BSA-treated glass

Unbinding force from BSA-treated polystyrol

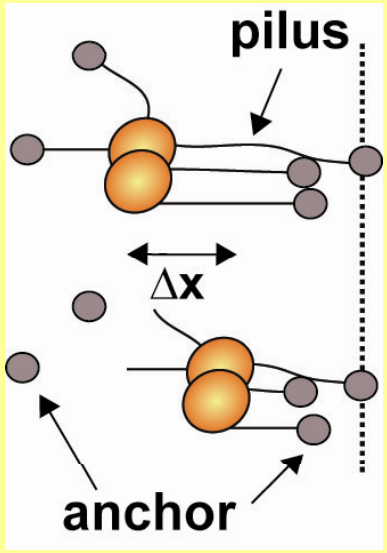
Stalling force of individual pilus motors

=> Unbinding force depends on surface and is significantly lower than stalling force

Conclusion

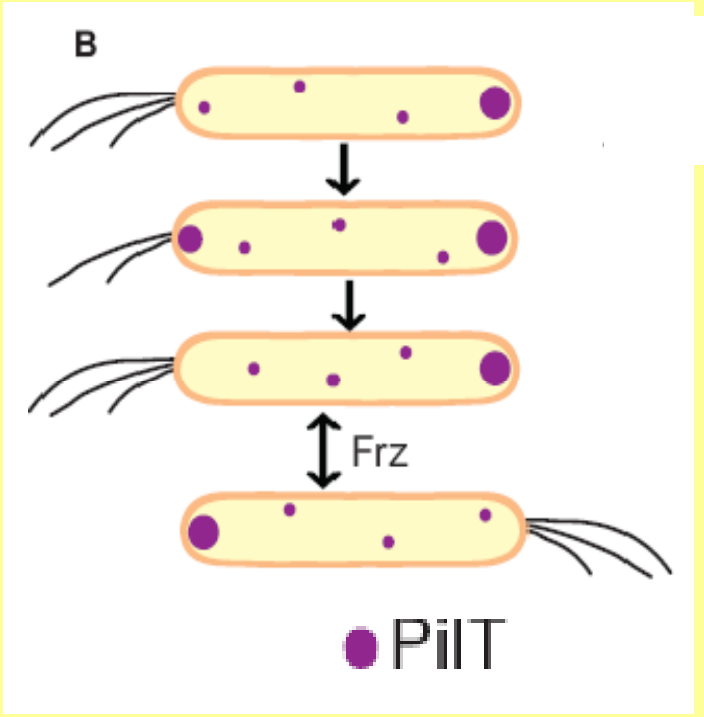
N. gonorrhoeae

- Multiple pili are required for persistent movement
- Unbinding force of pili from surface is significantly lower than motor force



=> consistent with tug-of-war

M. xanthus

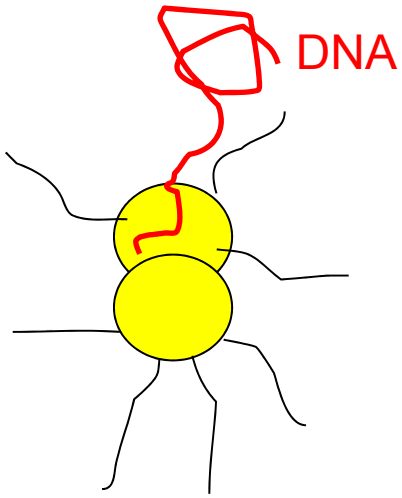


=> highly coordinated motor dynamics

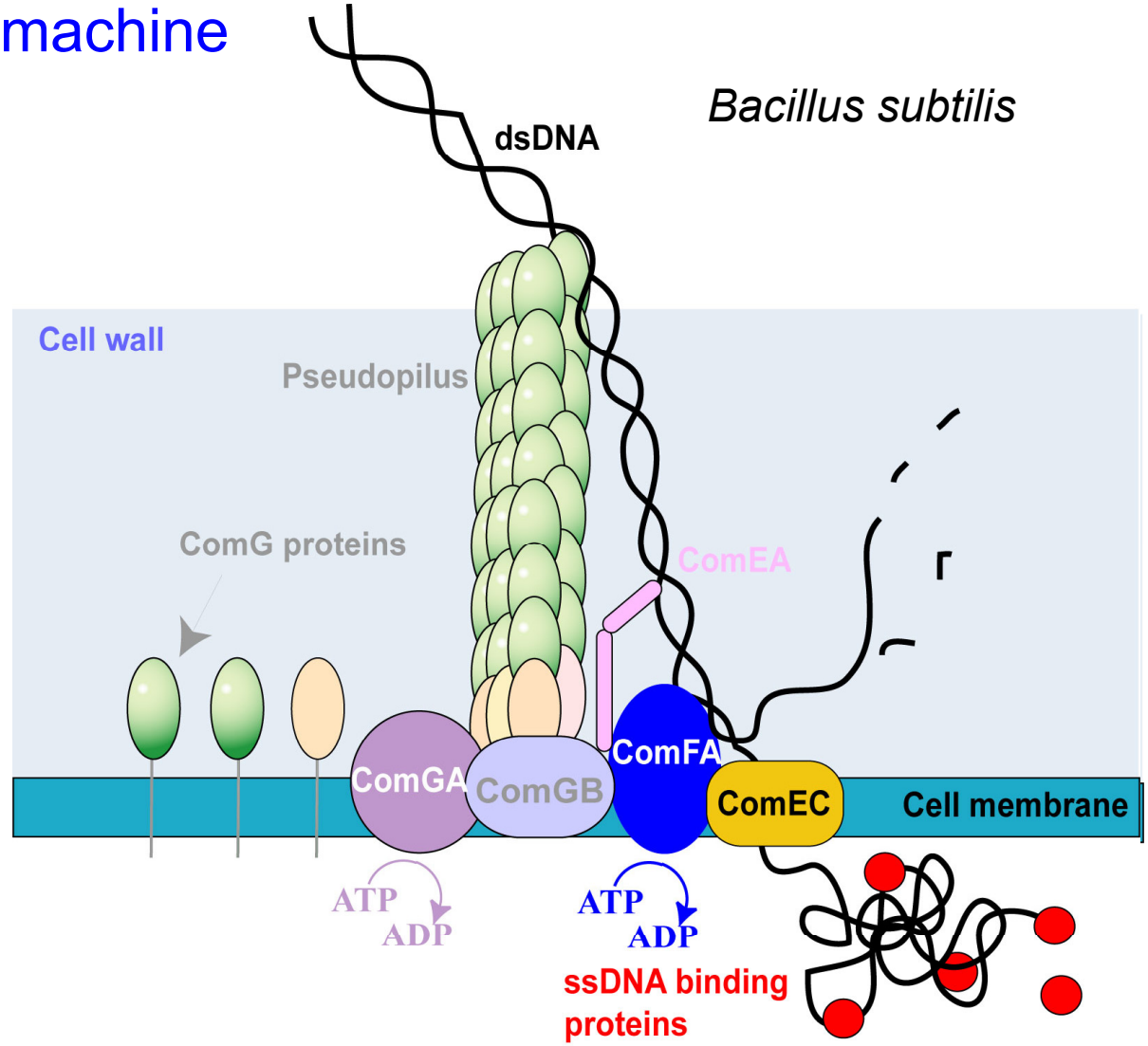
Bulyha, Schmidt, Lenz, Jakovljevic, Höne, Maier, Hoppert, Sogaard-Andersen, Mol.Microbiol. 2009
Holz, Opitz, Kurre, Greune, Schmidt, Maier, PRL, 2010

Different machines have evolved for DNA import

Do biophysical properties converge?



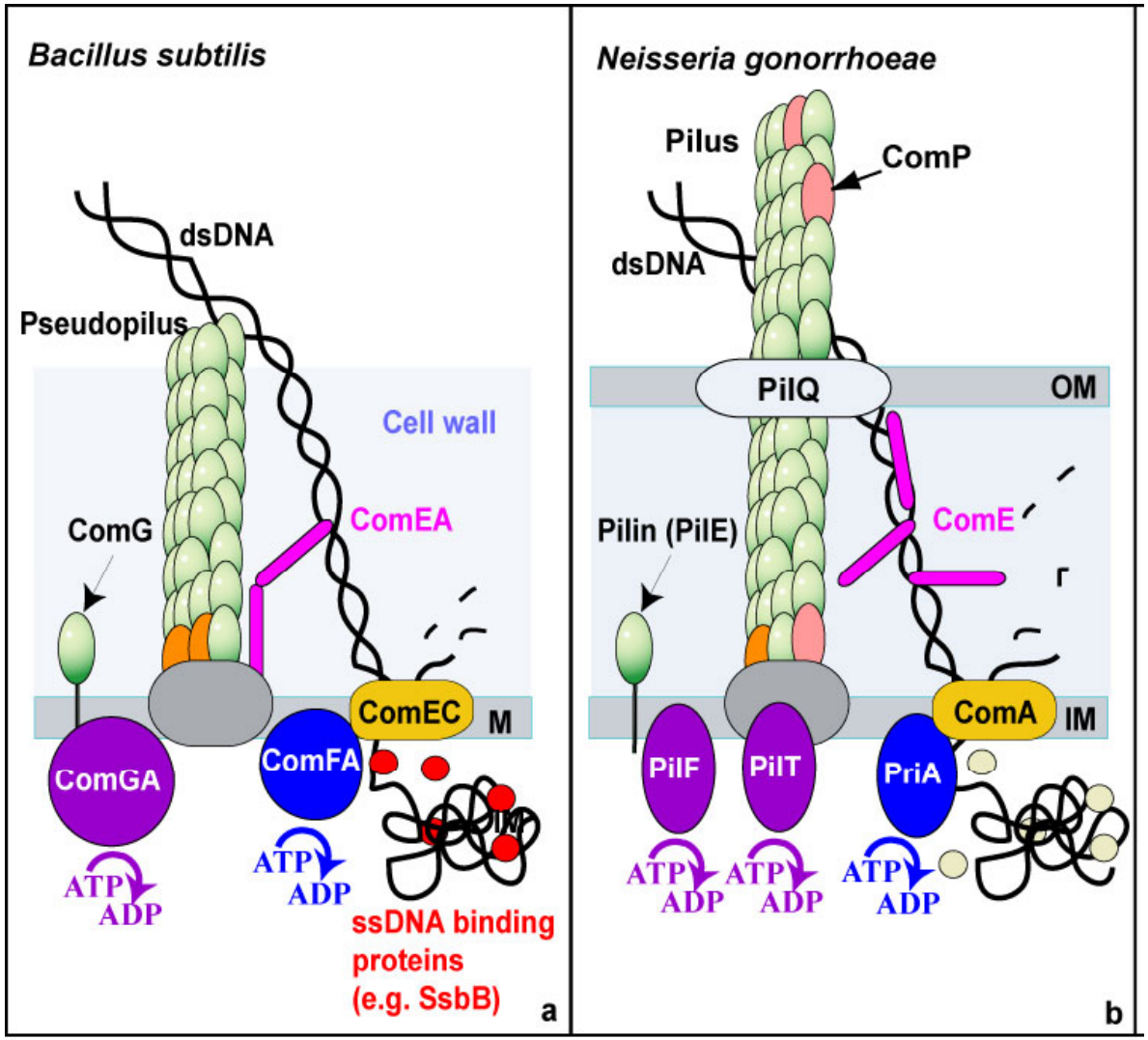
DNA import machine



adapted from Chen & Dubnau 2005

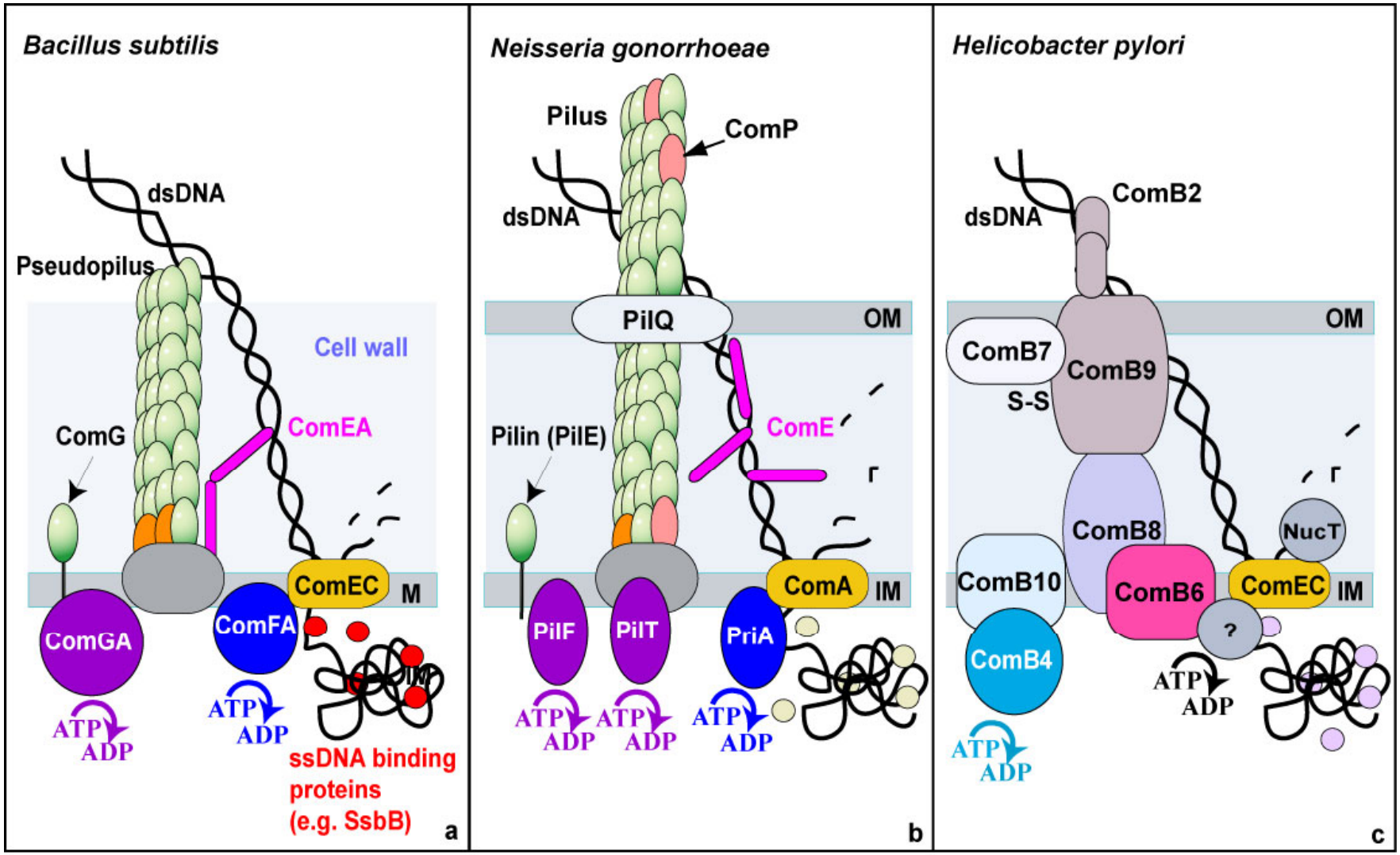
Different machines for DNA import

All naturally competent species use the T4 pilus for DNA import ....

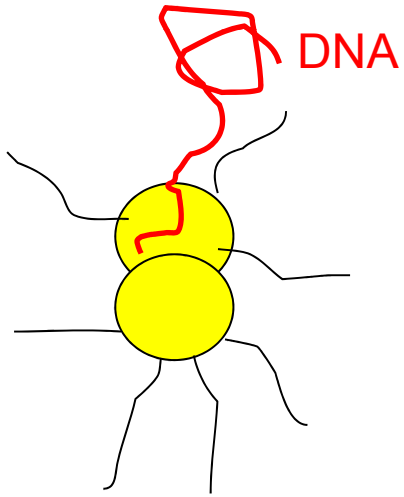


Different machines for DNA import

... with the exception of *Helicobacter pylori*



Molecular mechanism of the DNA import machine

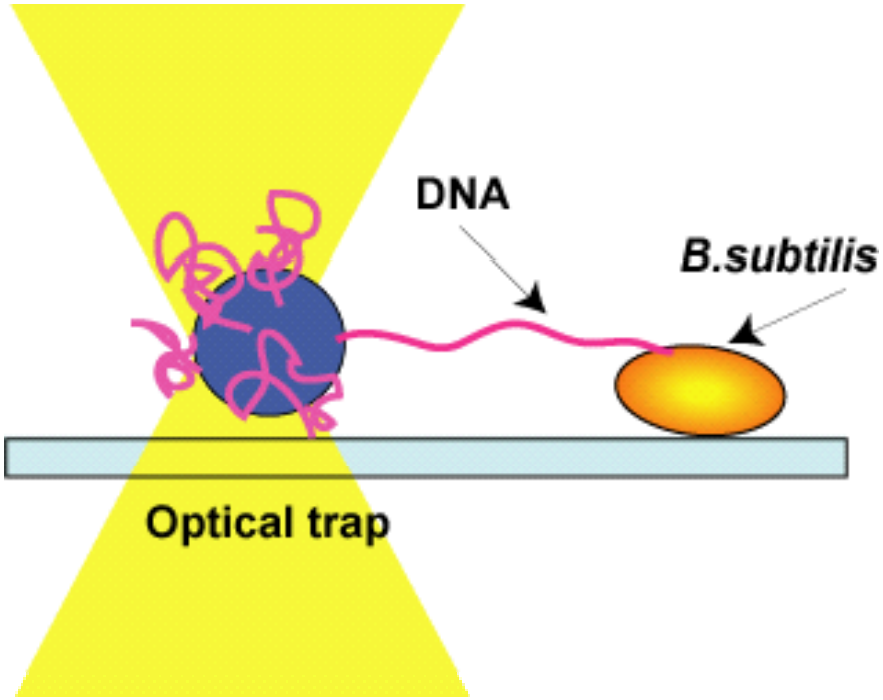


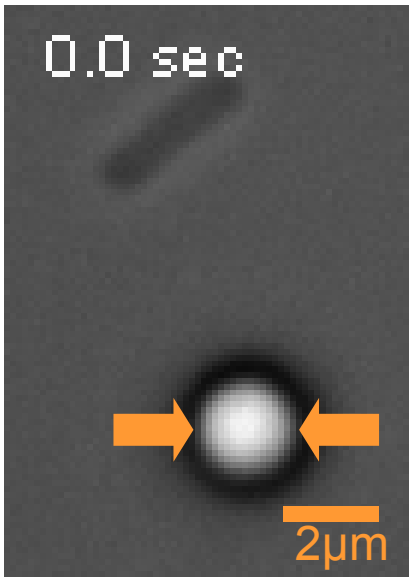
Kerstin Stingl, Stephanie Müller, Martin Clausen

David Dubnau, PHRI Newark

Michael Koomey, Oslo University

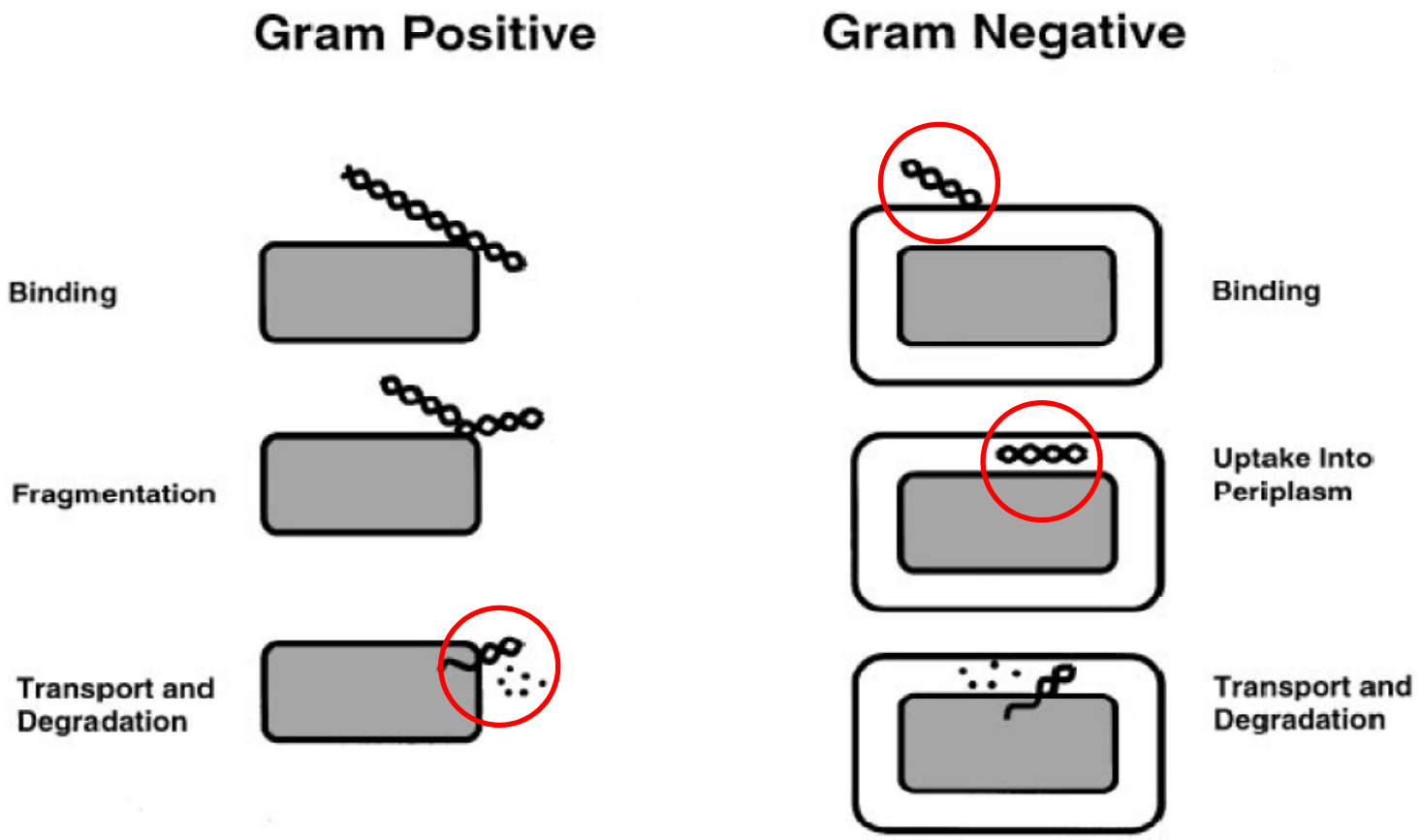
Import of individual DNA by *Bacillus subtilis*





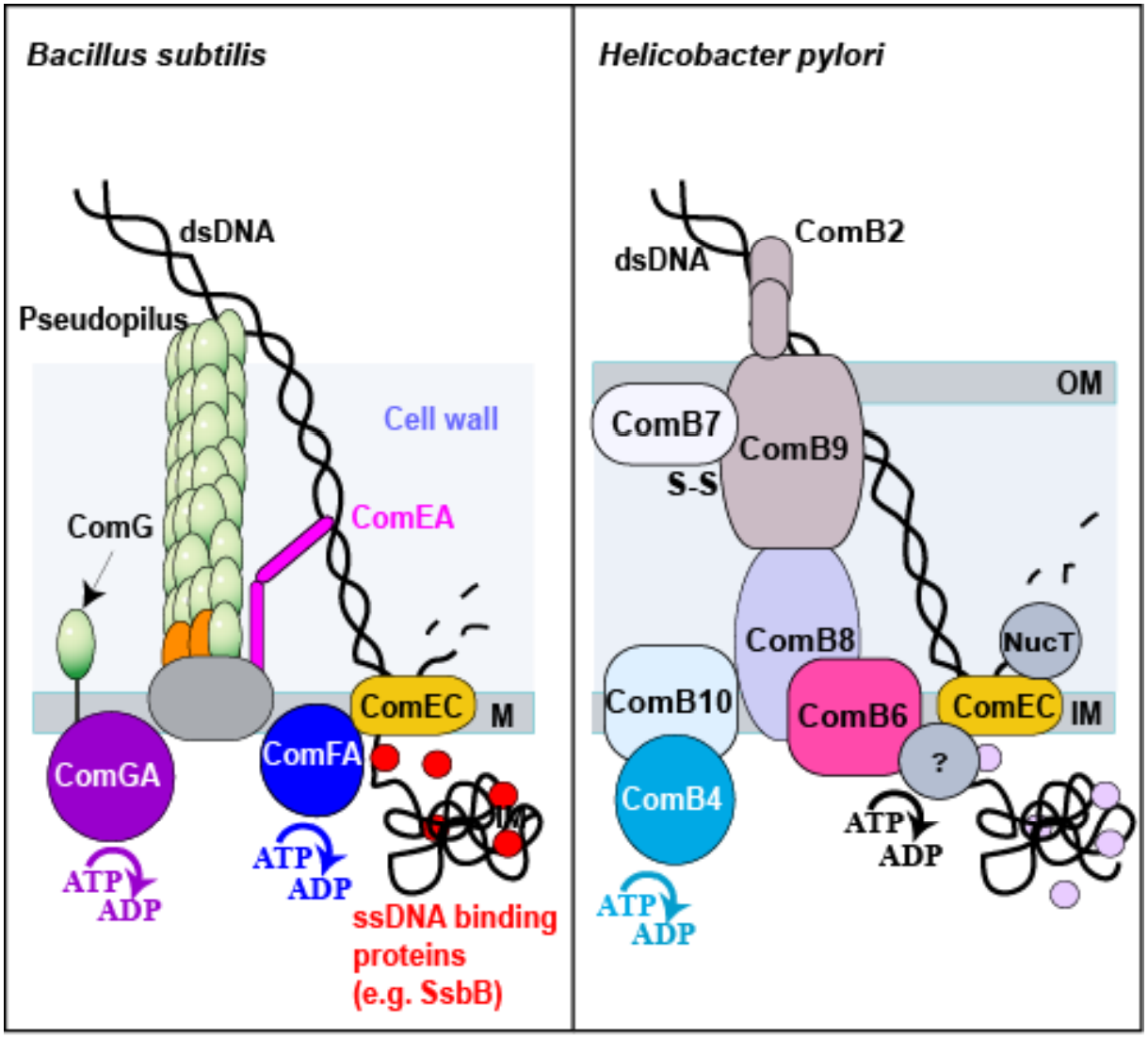
Optical trap

DNA import through two membranes



Dubnau, Annu. Rev. Microbiol., 1999

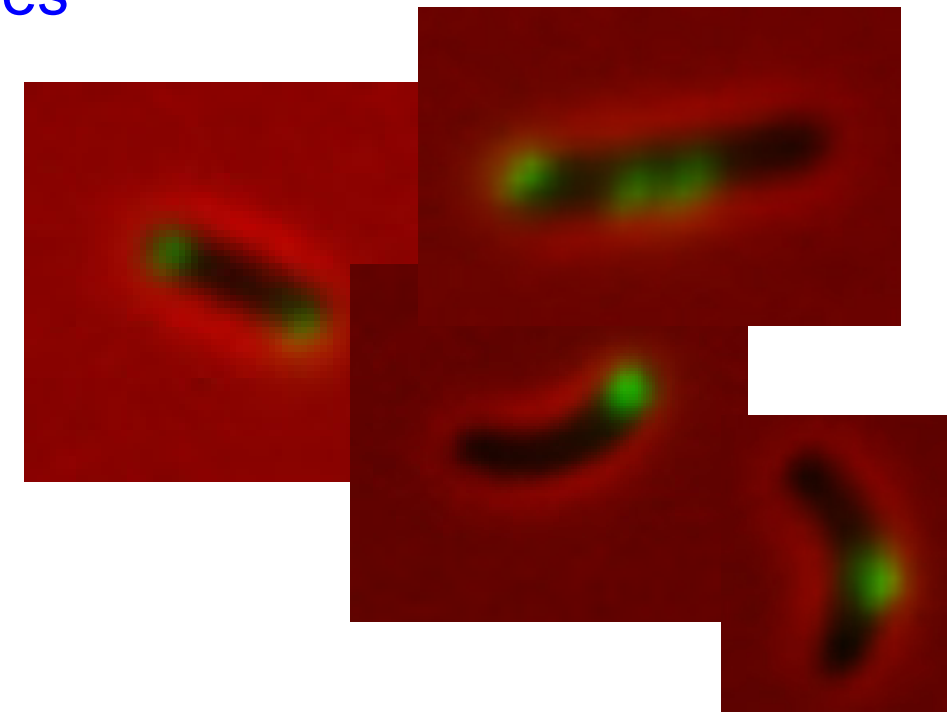
DNA import in *Helicobacter pylori*



DNA-uptake occurs from cell poles

Cy3-DNA-foci in DNAse treated

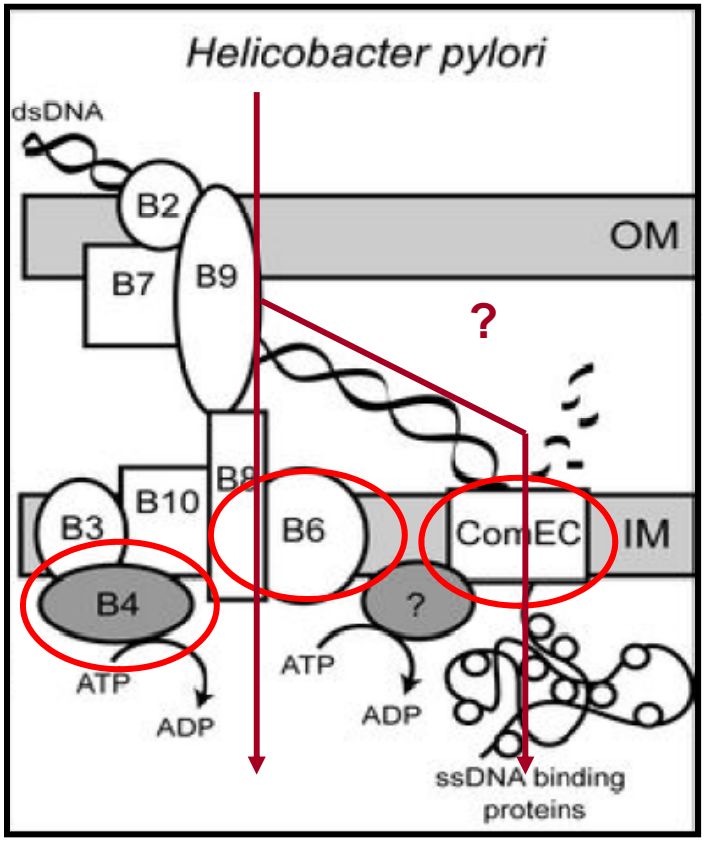
H. pylori:



- Polar localisation
- multiple foci possible
- parental strain: 74 ± 9 % of cells show foci

  (one focus: 32 ± 3 %, two foci: 26 ± 4 %, three or more foci: 16 ± 6 %)
- no distinct competence phase (stationary phase: 58 ± 2 %)

DNA-uptake depends on ComB system



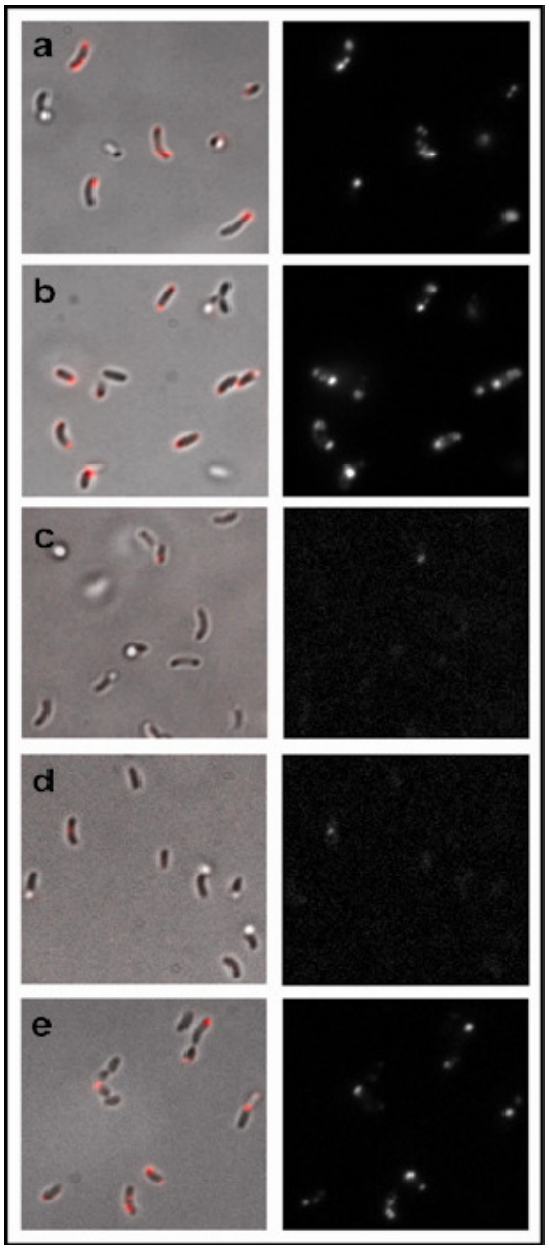
Parental strain

74% ± 9%

Parental strain

with 1kb λ-DNA

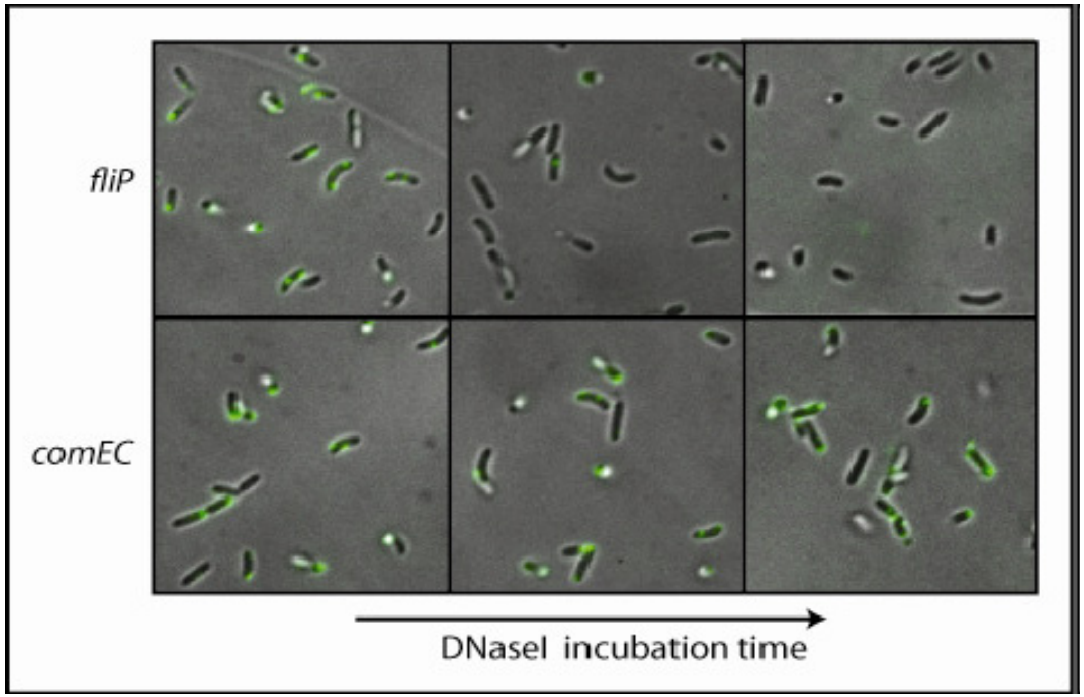
79% ± 7%



Temporal coupling of transport between outer and inner membrane

Foci disappear, but
time until foci vanish varies
(immediate or up to 60min)

Foci never disappeared
(not even after 90min)

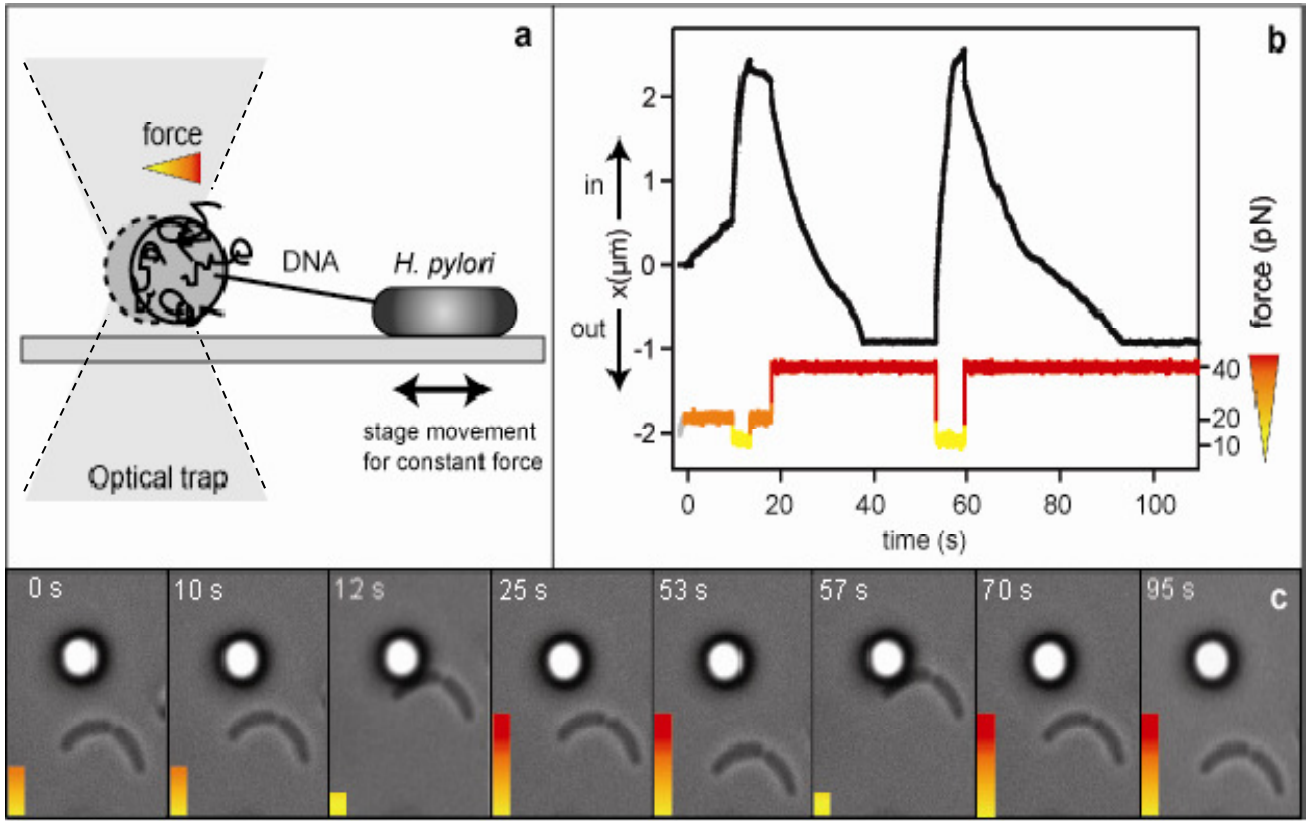


Green foci: imported fluorescently labeled DNA

=> DNA enters periplasm as dsDNA

=> inner and outer membrane transport are temporally uncoupled

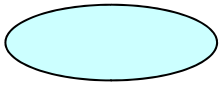
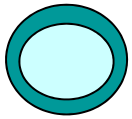
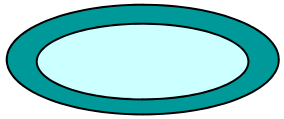
Kinetics and force generation during DNA-uptake in *Helicobacter pylori*



=> Import is reversible at 23 ± 3 pN

=> Average velocity @ 10pN: wt (*fliP*): v = 1.26 ± 0.46 kbp/s

Conclusion

	B. subtilis	*N. gonorrhoeae**	*H. pylori*
			
Velocity (10pN)	80bps/sec	80bps/sec	1.2kbps/sec
Maximum force	80pN	40pN	23pN
Reversibility	no	yes	yes
DNA specificity	high		weak for om, strong for im
Polar foci	yes	no	yes
Regulation of competence	quorum sensing nutrient limitation	DNA uptake sequence	none known

=> inner membrane transport conserved, outer membrane transport species-dependent

Maier, B., Chen, I, Dubnau, D., Sheetz, M. Nat.Struct.Mol.Biol., 2004
Hahn, Maier, Haijema, Sheetz, Dubnau, Cell 2005
Stingl, K., Müller, S., Scheidgen-Kleyboldt, G., Clausen, M., Maier, B., PNAS 2010
Stingl, K., Clausen, M., Maier, B. unpublished

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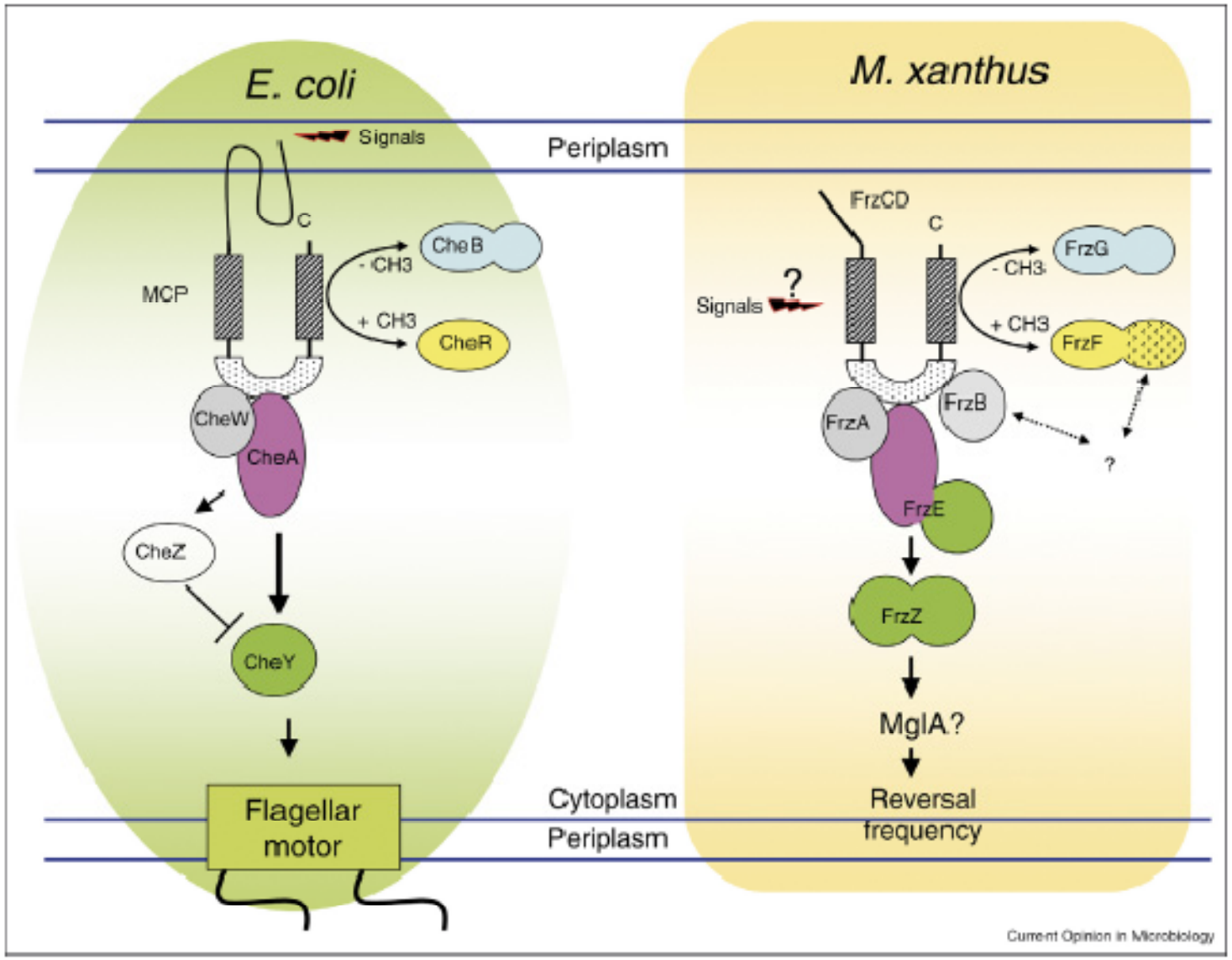
Cell Dynamics and Disease

GRK1409

The Frz system



Mauriello & Zusman, 2007

The Frizilator