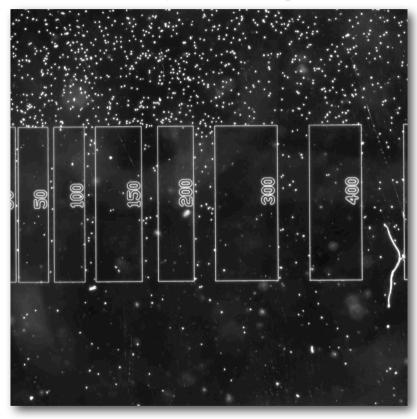
# Biophysics of swimming cells (selected topics)

E. coli

Turner, Ryu and Berg J. Bacteriol. 182, 2793 (2000)  $10 \, \mu m$ 

C. reinhardtii (eukaryotic microalga)



Marco Polin
Physics Department
University of Warwick (UK)

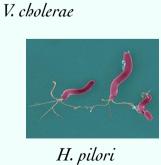
#### The Plan:

- Low-Re very quick recap
- Bacterial flagella
- · Run-and-tumble

- Eukaryotic flagella
- Flagellar synchrony
- Flagellar growth

## Prokaryotes and Eukaryotes:

two solutions to the swimming problem average width human hair  $\sim 10 \mu \mathrm{m}$  $\sim 100 \mu \mathrm{m}$ Eukaryotes protists choanoflagellates spermatozoa kinglab.berkeley.edu stanford.edu/group/Urchin eukaryotic microalgae



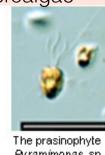
E. coli

 $\sim \mu \mathrm{m}$ 

Prokaryotes



Photo Seija Hällfors.

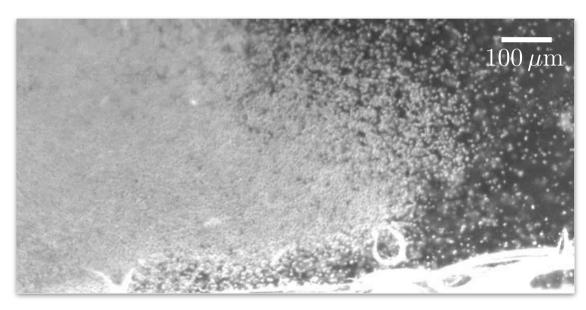


Pyramimonas sp. Scale bar 30 µm. Photo Seija Hällfors.

Brennen and Winet (1977)

Eukaryotes

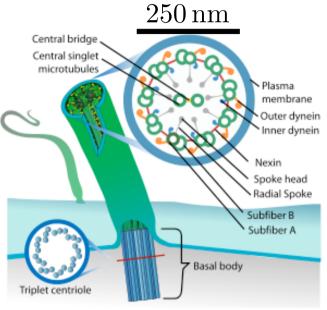
## Generally larger (>10µm) and faster (>100µm/s) than bacteria



Chlamydomonas photo-bioconvection (10x speed)

#### Eukaryotic flagellum/cilium

## sophisticated 250 nm



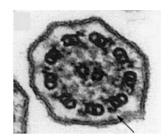
http://en.wikipedia.org

- transport
- propulsion
- mechanochemical sensing
- mating
- regulation of cell cycle

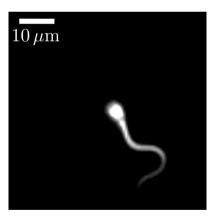
• . . . .

#### highly conserved

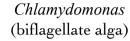
Homo sapiens

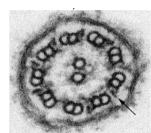


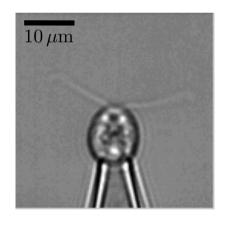
Pazour *et al.* (2006)



Prof. U. B. Kaupp, Forschungszentrum Jülich







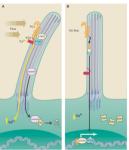


### Involved in a wide variety of ciliopathies

**Table 2**Ciliopathies, phenotypes, and associated genes.

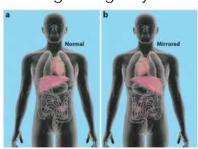
Disorder	Overall phenotype	Ciliary phenotype	Genes
Bardet-Biedl Syndrome	<ul> <li>Polydactyly</li> <li>Obesity</li> <li>Retinal degeneration</li> <li>Renal dysfunction</li> <li>Mental retardation</li> <li>Hypogonadism</li> </ul>	Truncated	BBS1-12
Nephrononphthisis Senor-Loken Syndrome	<ul><li>Cystic kidneys-reduced kidney size.</li><li>NPHP</li><li>Retinal involvement</li></ul>	Fewer, truncated Fewer, truncated	NPHP1-9, Nek8 NPHP1, NPHP4, NPHP5/IQCB1, NPHP6/CEP290, SDCCAG8
Joubert Syndrome	<ul><li>NPHP</li><li>Eye/Retinal involvement</li><li>Cerebellar Vermis Aplasia</li></ul>	Fewer, truncated	INPP5E, TMEM216, AHI1, NPHP1, NPHP6/CEP290, TMEM67, RPGRIP1L, ARL13B, CC2D2A, OFD1
Jeune Asphyxiating Thoracic Dystrophy Polycystic Kidney	<ul> <li>Skeletal abnormalities (small thoracic cage)</li> <li>Enlarged, cystic kidneys</li> </ul>	None (defects from abnormal hedgehog signaling) Fewer, truncated	IFT80
Disease Juvenile Cystic Kidney		Elongated	IFT88, PKD1, PKD2 Nek8
Disease Sensenbrenner	Cystic kidneys     Craniofacial abnormalities	Fewer, truncated	IFT22, IFT43
Syndrome	Cramoracian abnormanics	rewer, transacted	1122, 1113
Situs Inversus	<ul> <li>Randomization of visceral organ laterality</li> </ul>	Absent, Truncated, Immotile	Kif3A/B, NPHP2
Meckel-Gruber Syndrome	<ul><li>Cystic kidneys</li><li>CNS malformation</li><li>Hepatic abnormalities</li></ul>	Elongated	MKS1,3,5,6; CEP290, BD91,BD92
Tuberous Sclerosis	Tumors affecting brain, skin, heart, kidneys, and lung	Elongated	Tsc1, Tsc2
Alstrom Syndrome	Retinal dystrophy	Positioning defect, truncated (in some models)	ALMS1
	<ul> <li>Hearing loss</li> <li>Obesity</li> <li>Type 2 diabetes</li> <li>Short stature</li> <li>Reduced intelligence</li> </ul>		
Orofaciodigital Syndrome 1	Malformation of face, oral cavity, digits	Fewer	OFD1
Primary Ciliary Dyskinesia	<ul> <li>Impaired airway mucus clearance (chronic respiratory infections)</li> <li>Hydrocephalus</li> <li>Infertility</li> </ul>	Immotile	DNAI1, DNAH5, TXNDC3, DNAH11, DNAI2, KTU, RSPH4A, RSPH9, LRRC50
Kartegener Syndrome	<ul><li>PCD</li><li>Situs inversus</li></ul>	Immotile	

#### kidney proliferation



V. Singla, Science (2006)

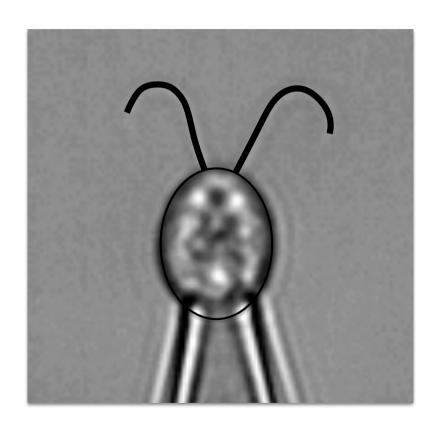
#### breaking left-right symmetry



Hirokawa N, et al. 2009. Annu. Rev. Fluid Mech. 41:53-72

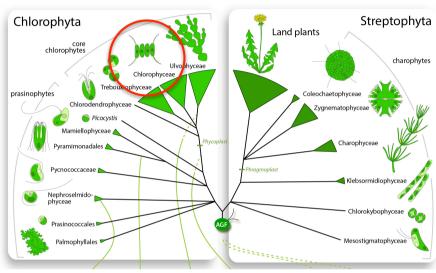
### We will focus on Chlamydomonas reinhardtii

- good model system to study flagella
- representative of eukaryotic swimming microorganisms



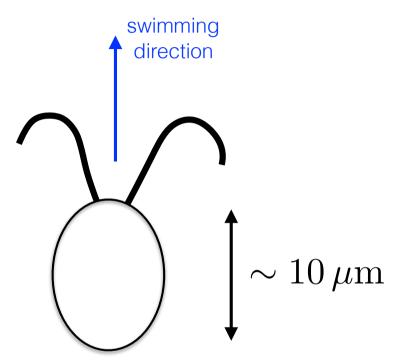
#### A quick tour of Chlamy

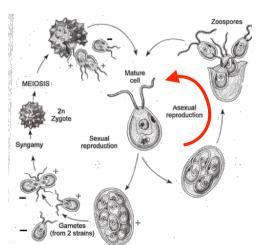
Unicellular biflagellate green alga
 (Volvocaceae ⊂ ... ⊂ Chlorophyta ⊂ Viridiplantae)



F. Leliaert, et al. Crit. Rev. Plant Sci. (2012)

- Cell body: ~10 μm; "front" flagella: ~12 μm / ~50 Hz
- Swimming speed: ~100 μm/s
- Cell spins at ~2 Hz
- Short vegetative life cycle (haploid):
   1 day, up to 8x growth
- 2 "mating types" (+/-) for sexual reproduction

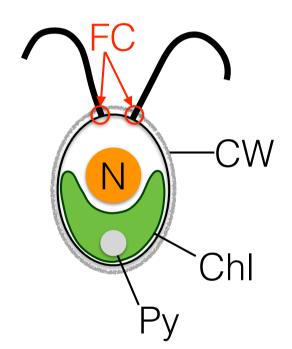




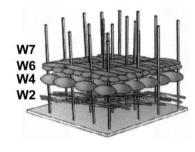
perrin33.com/microbiologie/lereste

#### A quick tour of Chlamy

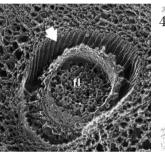
- Cell body surrounded by a cell wall (CW; ~200nm thick; glycoproteins; no cellulose)
- CW has specialised flagellar pores (Flagellar Collar; ~500nm Ø)

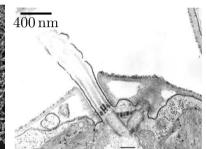


CW layers



Flagellar Collars





Chlamy Sourcebook

- Chloroplast (Chl) for photosynthesis (~70% cell volume)
- A single pyrenoid (Py): starch accumulation

Bioconvection!



M. Bees, University of York

biased upward swimming

$$\dot{\theta} = \frac{B}{\gamma_r} \sin(\theta)$$



gravitactic torque



bottom heavy

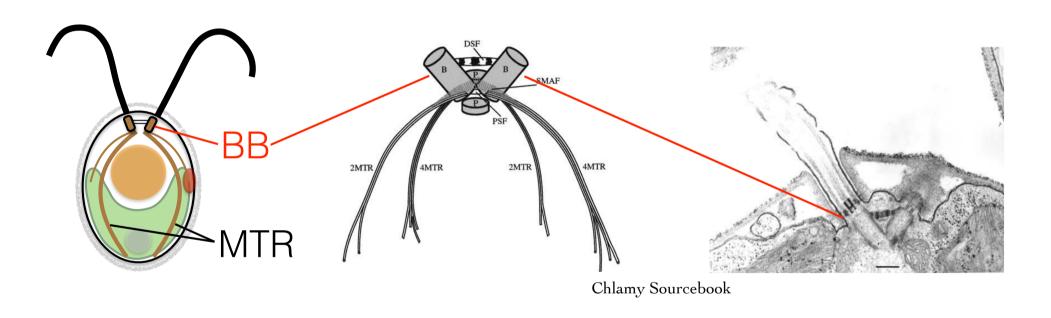


$$\mathbf{g} \quad \tau = B\sin(\theta)$$

#### A quick tour of Chlamy

 Mictorubule rootlets (MTR) ensure the correct internal organisation of the cell.

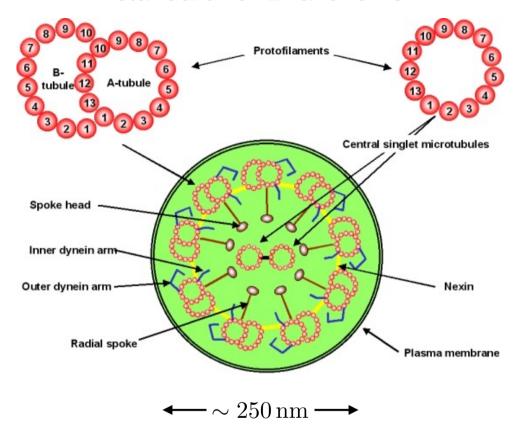
(E.g. the eyespot is ~20° ahead of the flagellar plane.)



- The rootlets stem from two Basal Bodies (BB):
  - Joined by fibres
  - Become centrosomes during cell division
  - Template the growth of flagella and root them in the cell body

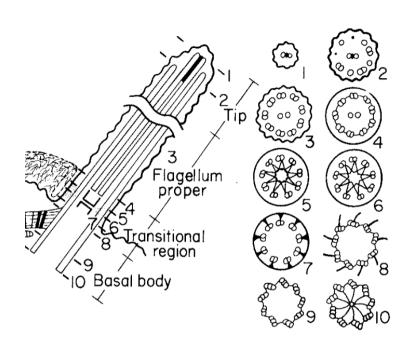
#### The structure: "passive scaffold"

#### standard "9+2" axoneme

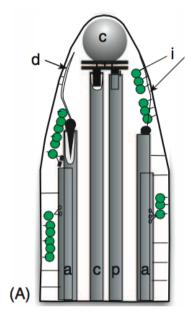


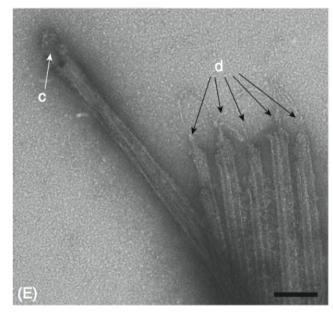
- 9 outer microtubule pairs (A and B)
- Nexin links between adjacent pairs
- Central microtubule pair (twisted in protists, not in animals)
- Radial spokes b|w central pair and outer doublets
- All microtubules plus ends towards the tip

#### The structure: "passive scaffold"



#### closeup on tip structures





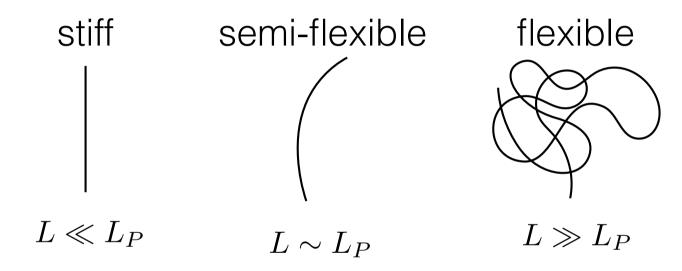
- Cilia ~2-5µm; Flagella ~10-20µm (typical)
- Connection to basal body by transition region (~200nm)
- Transition region is also the SOFA (site of flagellar autotomy) (wait a few slides)
- Specialised tip structure (~500nm)
- Specialised flagellar membrane: flagellar "necklace" controls mixing with cell membrane "proper"
- Ectosomes are constantly ejected at the tip (50-200nm): proposed to act e.g. as TxT Msg between cells

Question: are flagella stiff or flexible?

(you have 2 min to think about it)

### Stiff or flexible is a length-dependent concept

For "thermal" filaments: compare length L with persistence length L<sub>P</sub>



Q: Estimate the persistence length for a flagellum

$$L_P = \frac{EI}{k_B T}$$

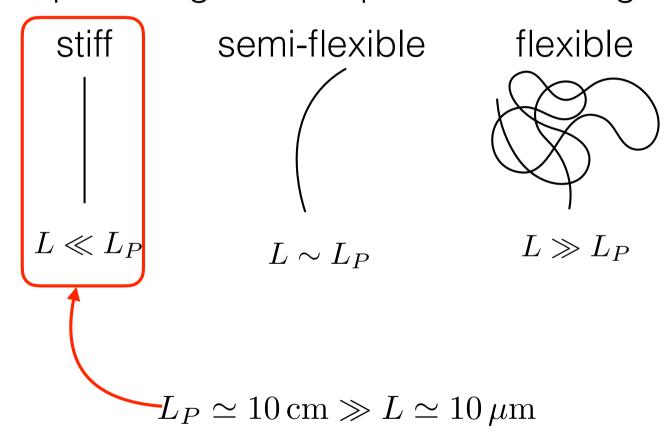
Flexural rigidity of a single microtubule

$$EI \simeq 2 \times 10^{-23} \, \mathrm{Nm}^2$$

Kikumoto et al. Biophys J. 2006

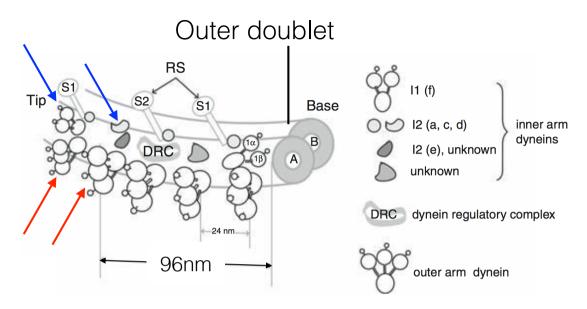
### Stiff or flexible is a length-dependent concept

For "thermal" filaments: compare length L with persistence length L<sub>P</sub>



...even without taking into account the internal structure!

#### The structure: "active components"



- 96nm repeat unit:
  - four outer dynein arms (oda): produce most of the power
  - various inner dynein arms (*ida*) + Dynein Regulatory Complex (DRC): mostly regulatory
  - Linker proteins b|w odas; idas & odas; DRC & odas (not shown)

How many dyneins are there in a typical 10um 9+2 flagellum?

Your Turn:

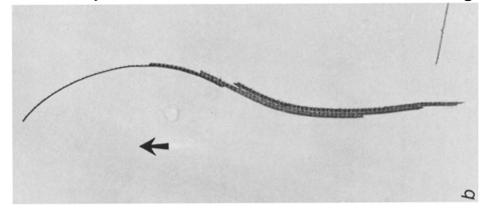
- Radial spokes (RS) approx. 30nm long:
- emerge from A µtubule, towards central pair at 16nm repeats;
- involved in regulating the activity of DRC and ida I1

~4500

#### The structure: "active components"

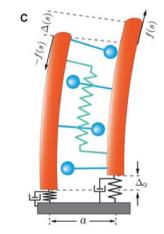
Isolated Tetrahymena cilia reactivated in ATP-containing buffer

 the dyneins generate relative translation between adjacent microtubule doublets



Warner & Mitchell, JCB (1981)

 Tangential shear from the dyneins is then converted to bending by the presence of mechanical constraints (basal body; nexins)



Riedel-Kruse I.H., et al. HFSP J. (2007)

- Periodic beating requires regulation of dynein activity
  - i) active clocking from the cell?
  - ii)emergent self-regulation within the axoneme?

#### The structure: "active components"

Sea urchin sperm

de-membranated and alive in "proper" buffer time

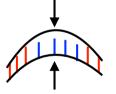
Gibbons & Gibbons, JCB (1972)

Axonemal beating is an "emergent property"

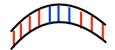
of the interaction between the passive scaffold and the active component (dyneins)

Currently three alternative hypotheses:

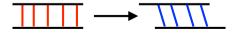
geometric clutch



curvature control

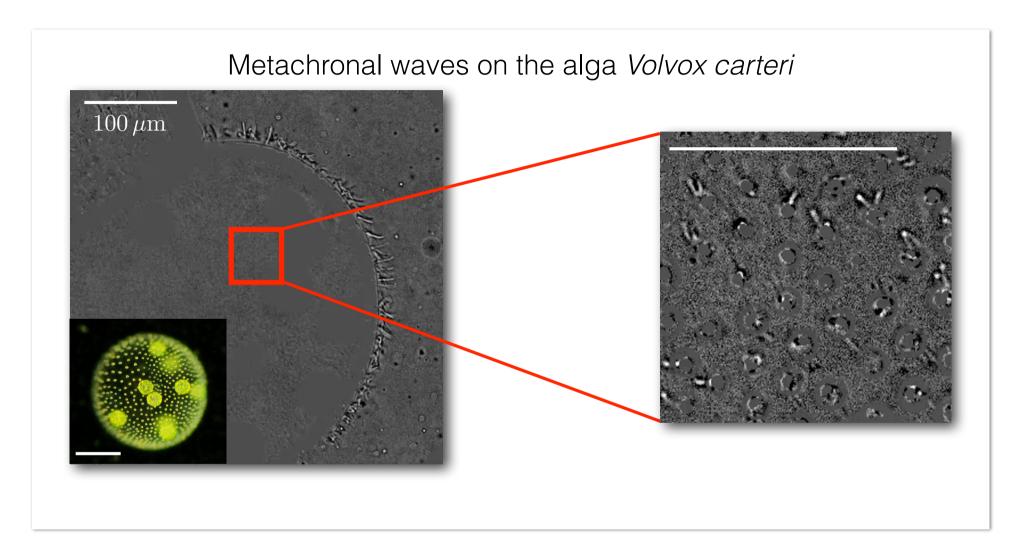


sliding control



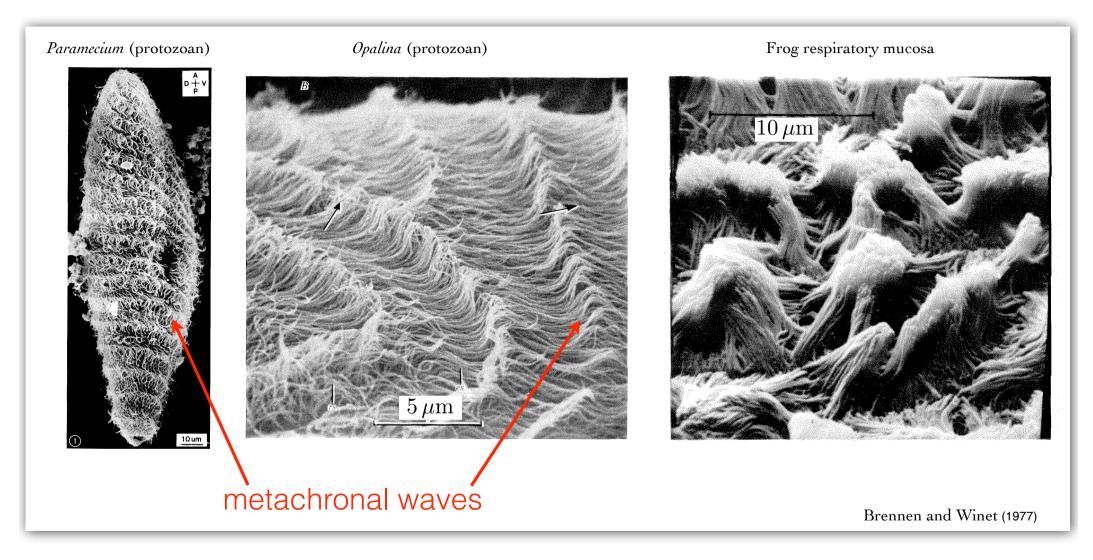
Interested? see e.g. notes in mpolin.com/teaching and outreach

# An unexpected consequence of motility: Flagellar Coordination



Coordinating thousands of eukaryotic flagella without "a brain"!

#### Flagellar coordination in eukaryotes: a universal feature

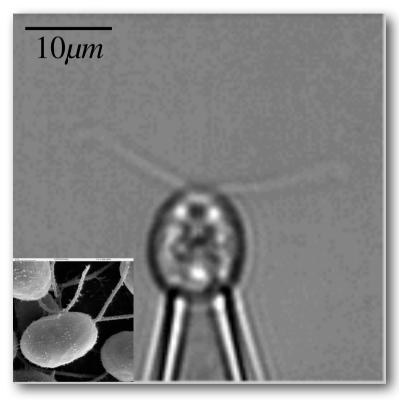


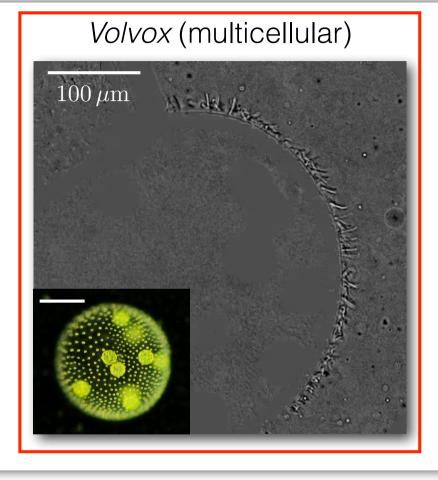
Can coordination emerge spontaneously?
Role of mechanical coupling?

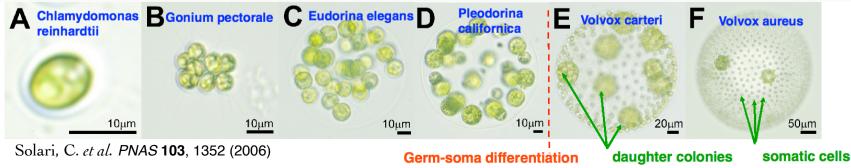
poorly understood

### Volvocales to the rescue: A model system for flagellar dynamics

#### Chlamydomonas (unicellular)



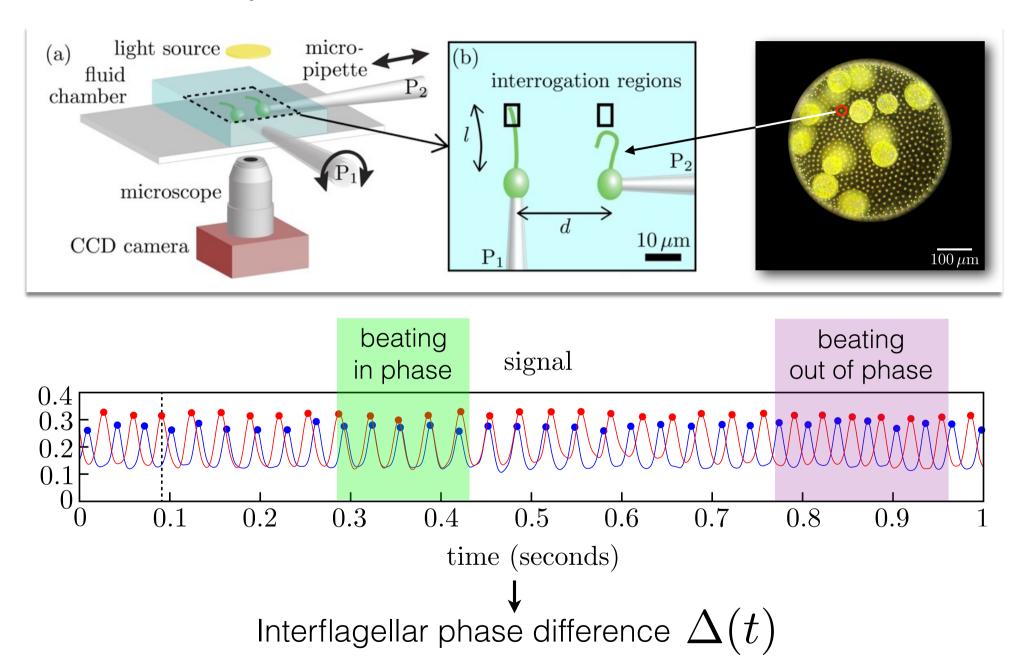




Kirk, D. L. Volvox, CU Press (1998); Kirk, D. L. BioEssays 27, 299 (2005); Herron M. D., and Michod, R. E. Evolution 62, 436 (2007); ....

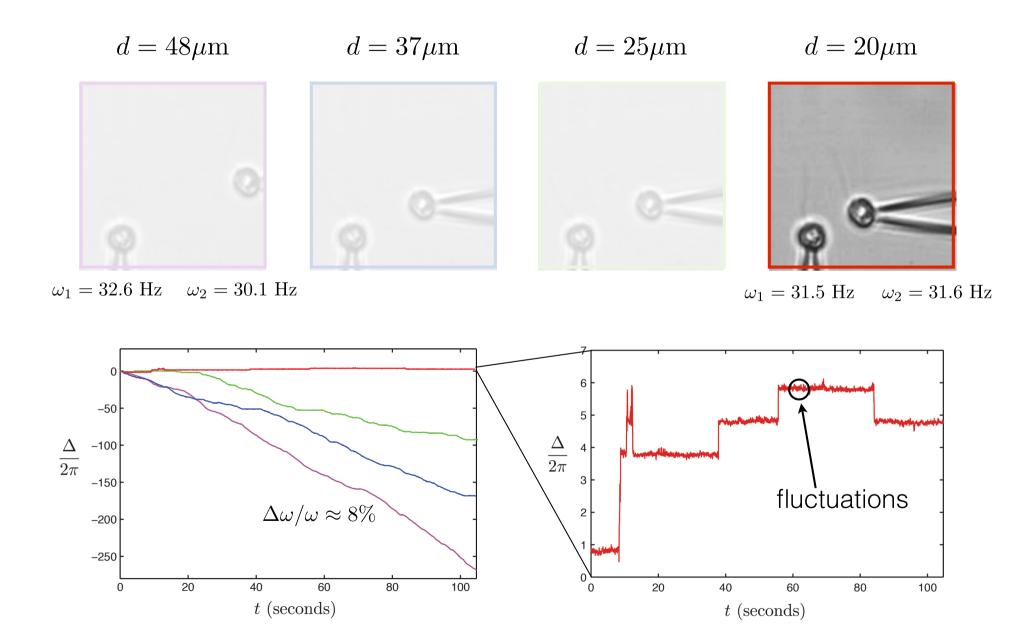
Coordination between two cells

#### Dynamics of individual somatic cells

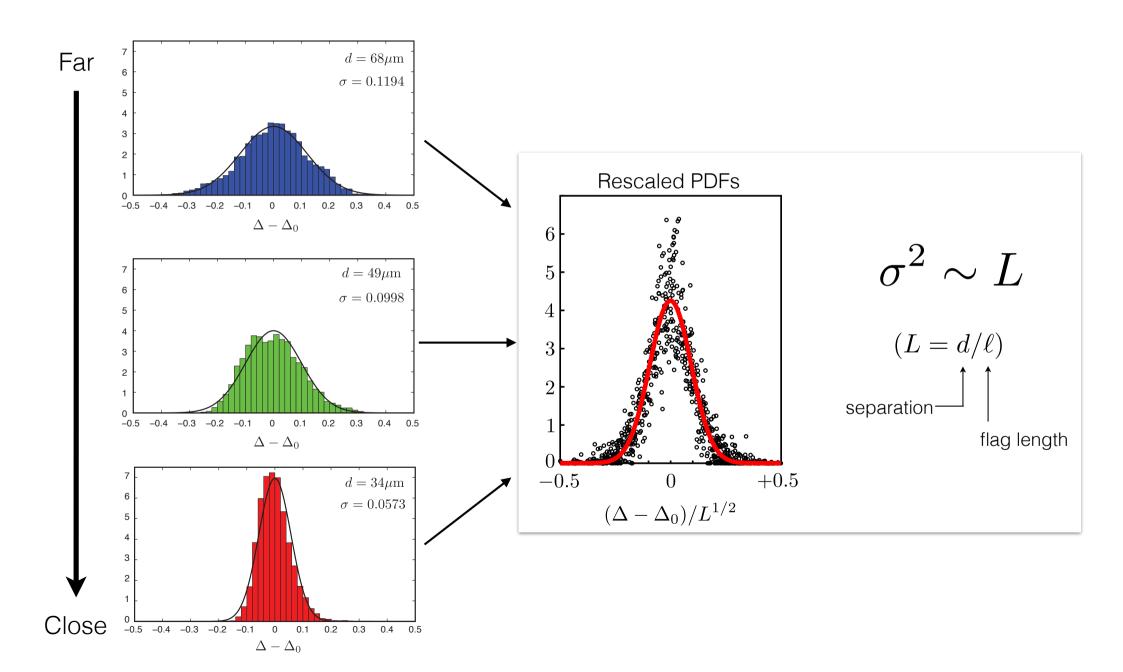


Analysis of experimental phase difference: Polin, et al. Science (2009); Goldstein, Polin, Tuval PRL (2009,2011); Leptos, et al. PRL (2013)

#### Synchronisation properties

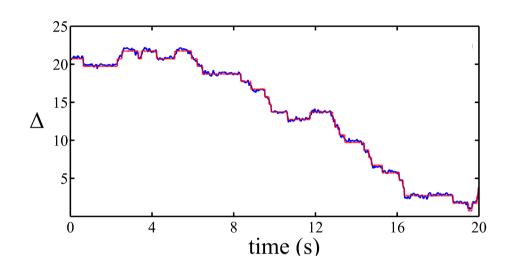


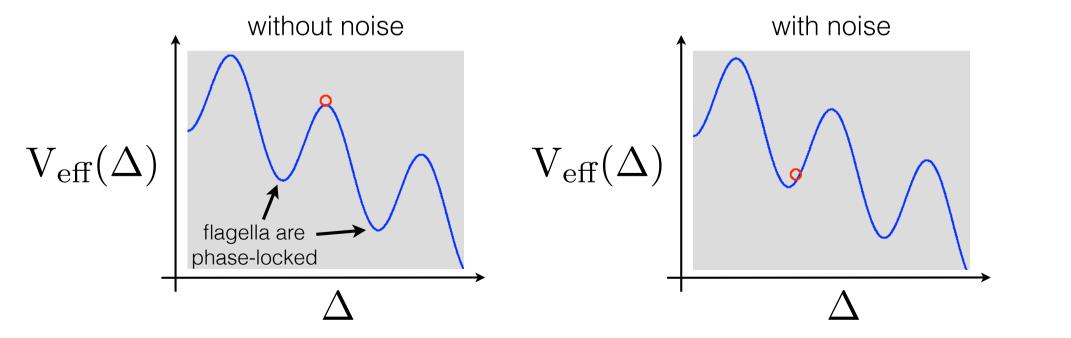
#### Fluctuations' PDFs during synchrony



#### Phenomenological model

Stochastic Adler model for  $\Delta(t)$ 

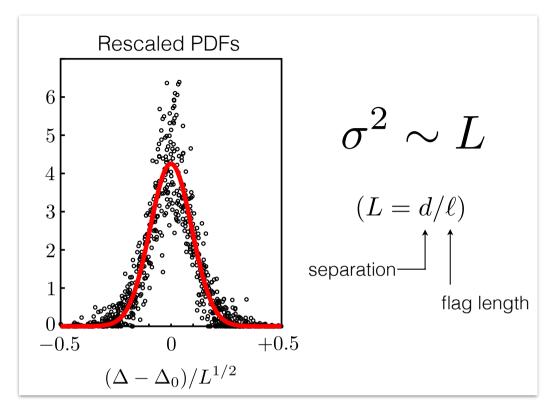




R. E. Goldstein, M. Polin, I. Tuval, PRL (2009)

#### Phenomenological model

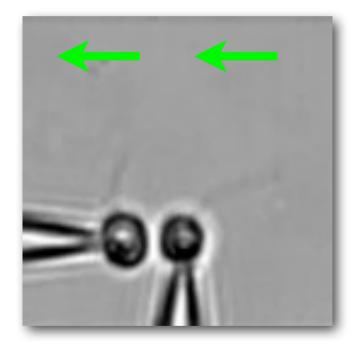
Stochastic Adler model for  $\Delta(t)$ 



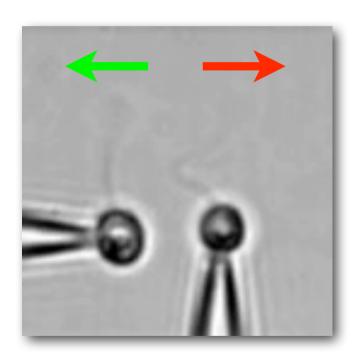
Q: Given the scaling of  $\sigma^2$ , how does  $\kappa$  depend on L??

### Dependence on alignment

In-phase



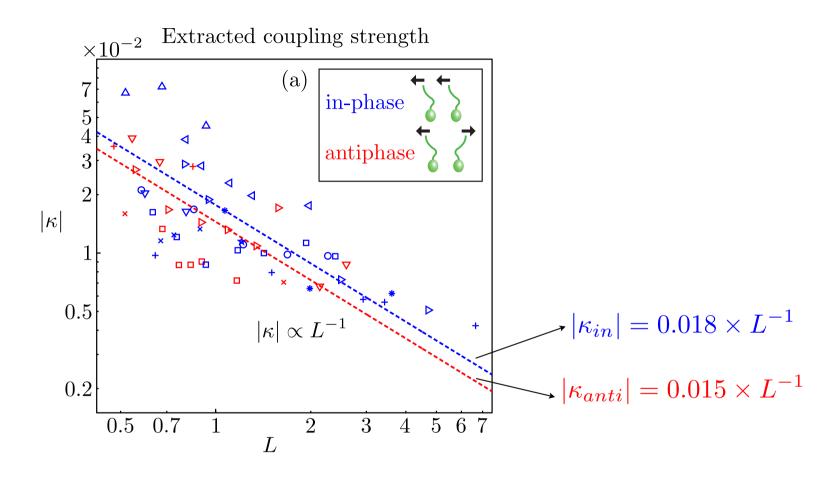
Anti-phase



$$\kappa > 0$$

$$\kappa < 0$$

#### Estimated coupling strength vs. separation

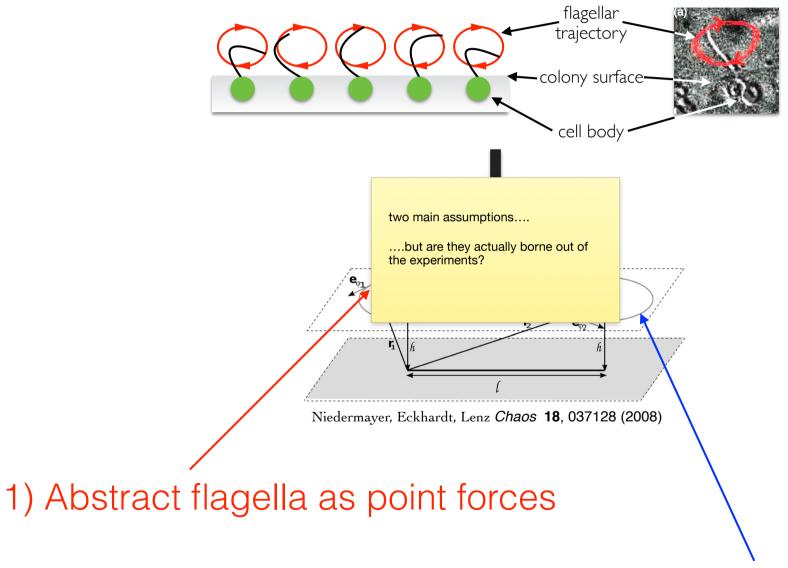


Experimental averages

Flag. length 
$$\langle \ell \rangle = 20.1 \, \mu \mathrm{m}$$
 } + Minimal model  $\longrightarrow |\kappa_{model}| = 0.015 \times L^{-1}$  Beating freq.  $\langle \bar{\nu} \rangle = 33.9 \, \mathrm{Hz}$ 

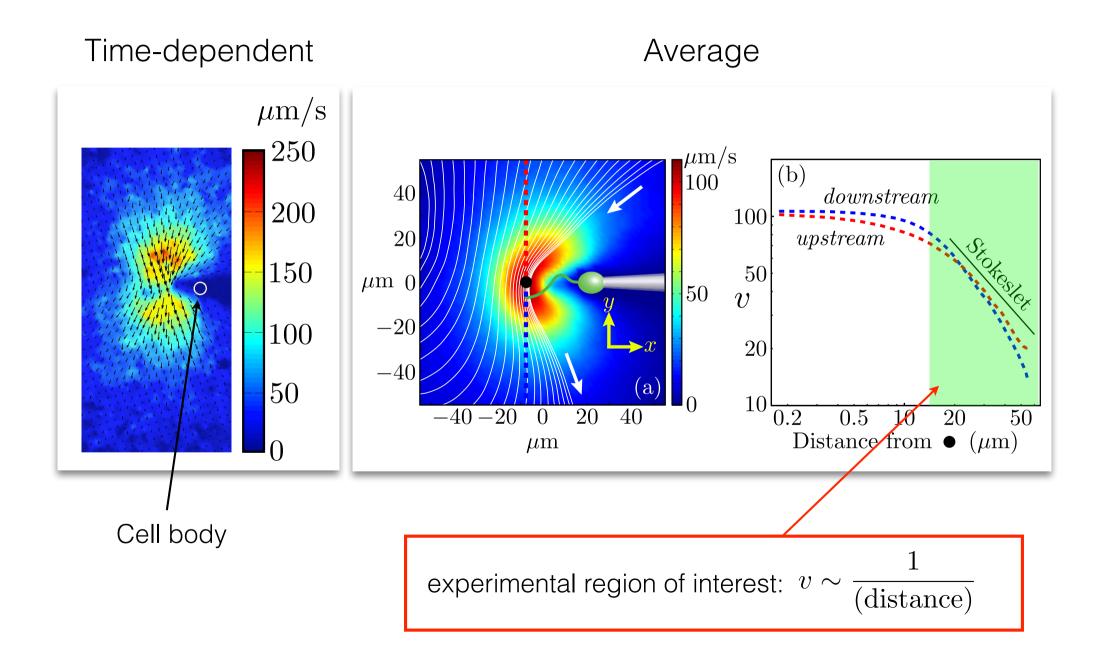
- Niedermayer, Eckhardt, Lenz *Chaos* **18**, 037128 (2008)
- Brumley, Polin, Pedley, Goldstein PRL 109, 268102 (2012)

## Minimal model: flexible rotating spheres

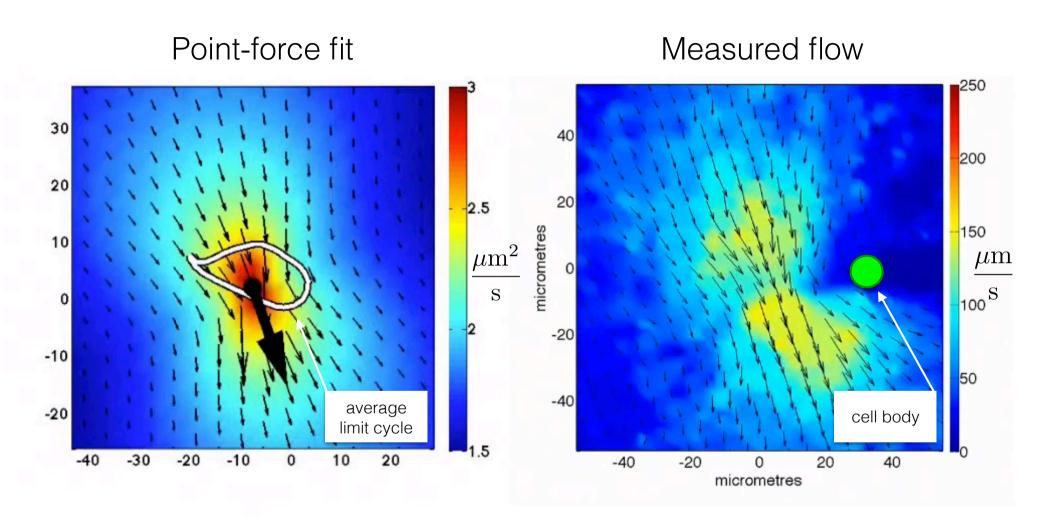


2) Assume that orbits are flexible

#### Single cell flow field $\,v\,$ is like that of a point force

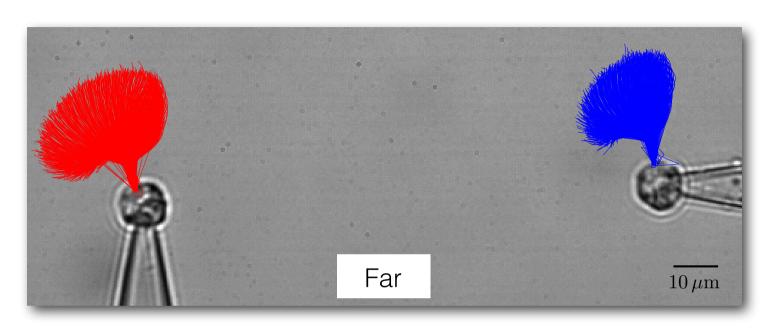


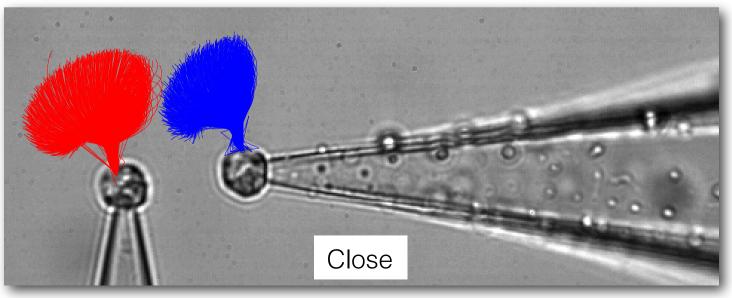
#### Point-force approximation to single cell flow



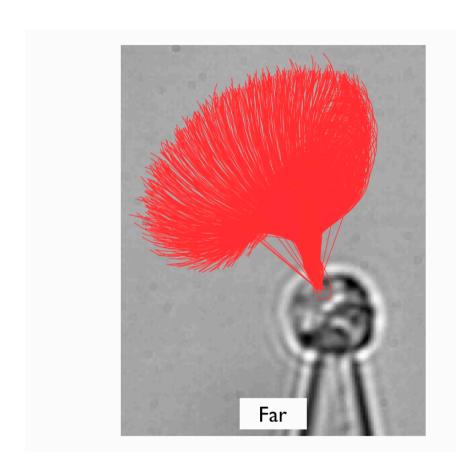
Well described by a point force but applied to a point that does not move along direction of the force (anisotropic drag of flagellum)

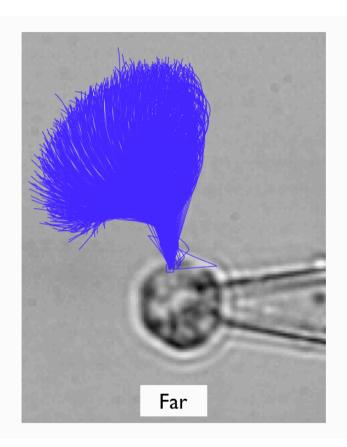
## Flagellar waveform vs. separation: flow induced deformations??





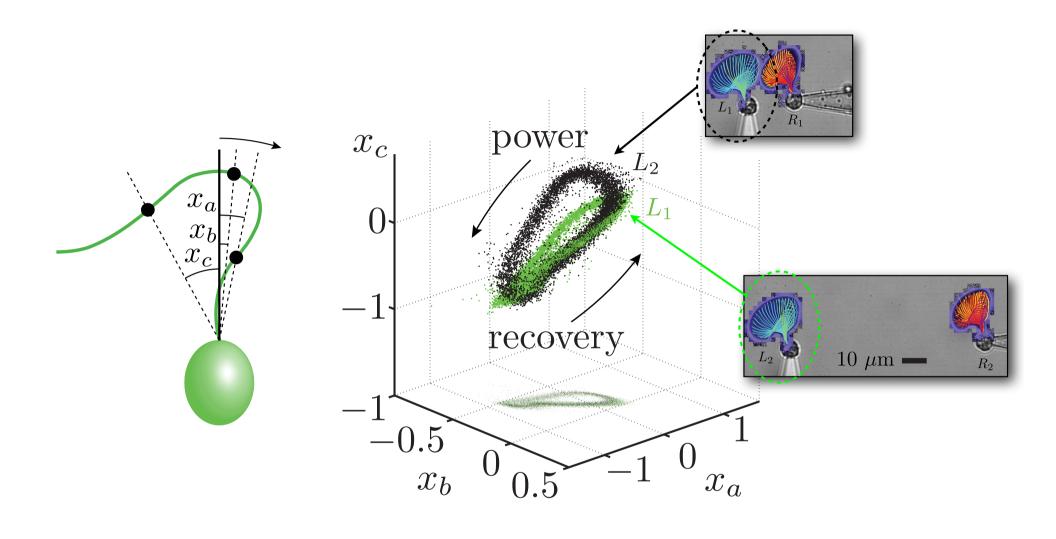
#### Flagellar waveform vs. separation





The waveform is distorted by the interaction

#### Interactions distort flagellar waveform



- •limit cycle constructed from 3 reference points along flagellum
- •approximately 8000 beats for each cell-cell separation
- •deformation is more pronounced along distal portions of the flagellum

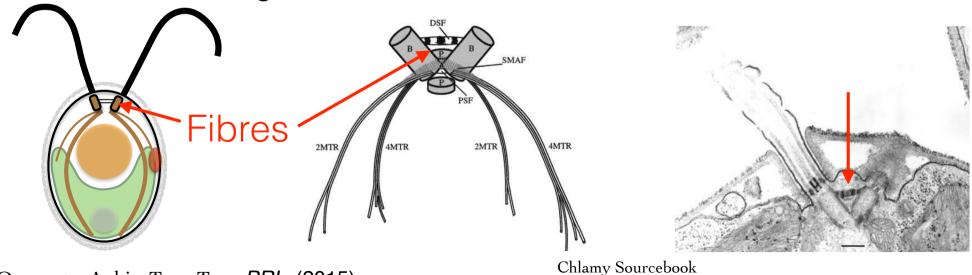
#### ...to summarise:

- Hydrodynamic coupling leads to flagellar synchronisation between different cells.
- •Synchronisation is accompanied by a subtle but easily measurable deformation of the beating waveform.
- Quantitative agreement with a simple minimal model supports coordination as an emergent property, resulting from the interplay of hydrodynamics and flagellar elasticity.

Done! We found the mechanism!!

...not quite!

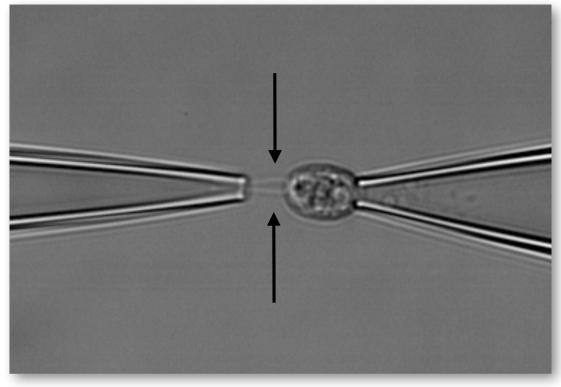
Within a single cell the mechanism seems to be different...



- •Quaranta, Aubin-Tam, Tam, PRL (2015)
- •Wan, Goldstein PNAS (2016)

Final stretch: Flagellar assembly!

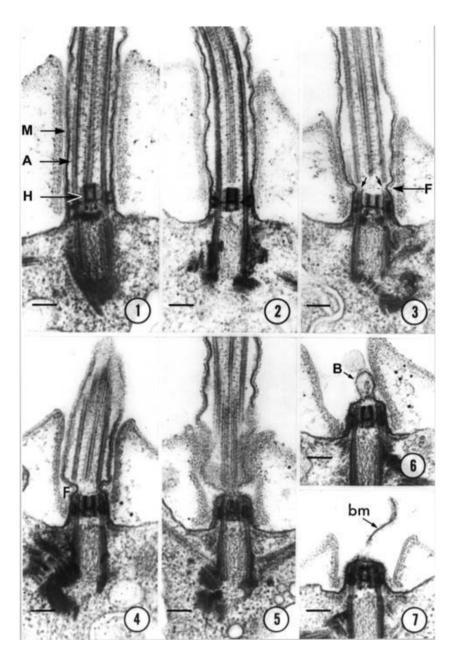
#### Flagellar Autotomy



R. E. Goldstein, M. Polin, I. Tuval PRL (2011)

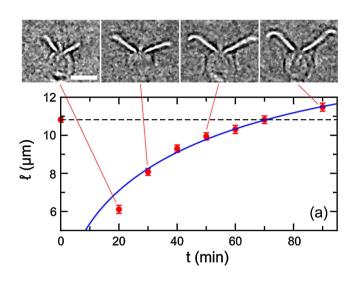
- Deflagellation in response to "harsh" stimuli: mechanical stress; pH shock; etc.
- Triggered by Ca<sup>2+</sup> influx
- A regulated process:
  - axoneme severed at the SOFA ("site of flagellar autotomy") within the transition region
  - produces a clean cut
- Unclear evolutionary advantage
- Instrumental for flagellar proteomics: easy to isolate and collect a lot of flagella

### How to cut a flagellum



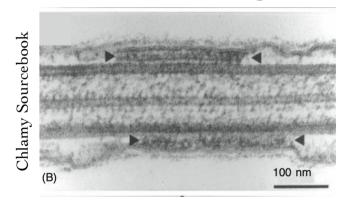
• If you shed it, you need to be able to GROW IT BACK

#### Flagellar regrowth

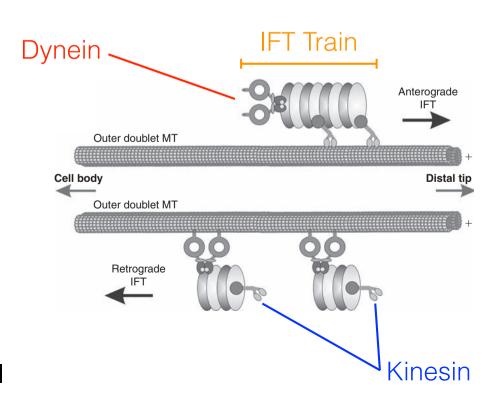


- ~1 2h to full length
- Non-linear growth dynamics
- Symmetric: same for both flagella
- Beating resumes at ~4µm length (why??)

#### Intra Flagellar Transport (IFT) (Highly Conserved!!)



- IFT Trains: 0.05-1µm long; 50nm wide
- Walk on B-microtubules of outer pairs
- Kinesin → Anterograde (tip-wise) ~2µm/s
- Dynein → Retrograde (BB-wise) ~3µm/s
- New proteins moved to tip; old go back to cell



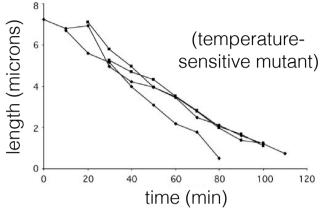
#### Reaching a finite length: the balance point model

IFT does not stop at full flag. length...

Outer microtubule doublet:
B tubule A tubule

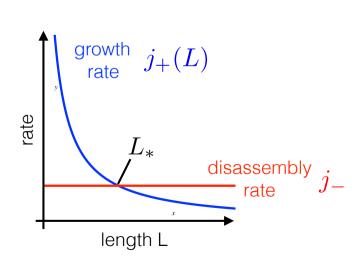
H. Ishikawa, and W. Marshall (2011)

Without IFT: flag. dissociates at constant rate



W. Marshall, et al. Mol. Biol. Cell (2005)

Length dependent growth + constant decay



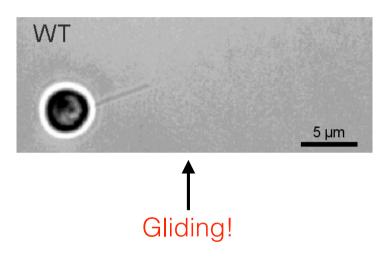
ecay 
$$\frac{dL}{dt}=j_+(L)-j_-$$
 
$$j_+(L)=p_{cargo}\,p_{int}\frac{\lambda}{\tau}$$
 
$$\tau=2L/Mv \qquad \text{IFT arrivals}$$
 constant # IFT trains

$$L_* = Mv\lambda\,p_{cargo}\,p_{int}/2\,j_-$$
 equilibrium length

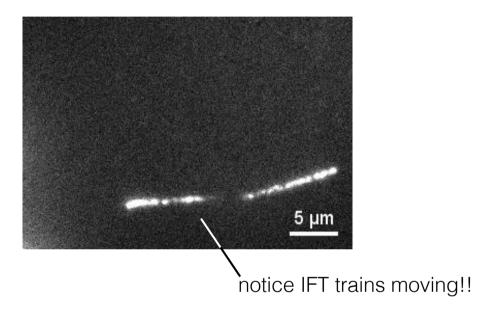
#### Gliding: an unexpected consequence of IFT

S. M. Shih, et al. eLife, 2, e00744 (2013)

## Chlamys attach on glass surfaces spreading their flagella



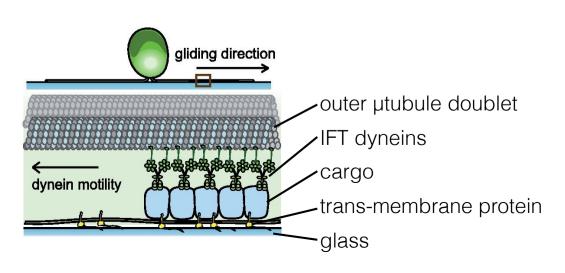
TIRF + GFP-tagged IFT trains



#### How gliding works

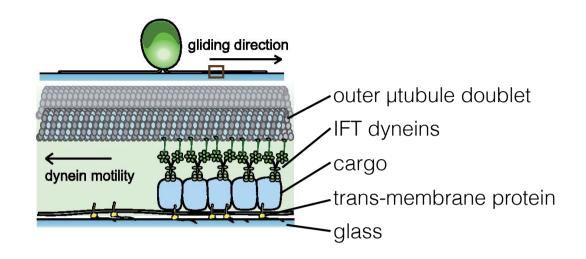
#### Retrograde dyneins:

- 1) attach to glass (transmembrane proteins) and....
- 2) pull the cell



#### Your turn

- gliding force ~20-30 pN
- gliding speed ~1-2 μm/s



How many dyneins are involved?

~4

Is the gliding velocity limited by viscous drag by the cell body?

No. It is not always hydrodynamics!