HEART OF THE (FLAGELLUM) BEAT

BY DR KIRSTY WAN (2014)

In the eye of the Sceptical Physicist, stochastic oscillators are found everywhere: in the periodic stroking of an oar, the rhythmicity of the human heartbeat, or even the beating flagellum of a swimming microorganism… Their noisy oscillations, and mutual interactions, turn out to be key physiological signatures of viability and health.

On a customarily mild spring day, you lean against one of the numerous bridges straddling the river Cam, and observe the passing of a coxed eight. It is a familiar spectacle, in which the flustered cox attempts to coax his team into stroking in unison. You identify one of the rowers, perhaps by the distinctiveness of his gear – a peculiarly fetching shade of pink, who is puffing his cheeks and taking in large gulps of air. Lining his respiratory system at this very moment, arrays of epithelial cilia work tirelessly to sweep away unwanted dirt, mucus, and irritants from his lungs. Just like a team of rowers, these tiny hair-like organelles must coordinate their rhythmic beating motion to drive physiological fluid flows, except…here there is no cox!

Together with that unsuspecting swan, various species of pond scum and algae are also pushed aside as the rower's blades part the water. These microorganisms can be brought into vivid focus under the scrutiny of a microscope, and found swimming, rolling, and 'cavorting in all directions' [van Leeuwenhoek; letters to the Royal Society, 1674]. Many of these cells have surface-attached flagella that are structurally very similar to lung cilia, but which serve as microscale analogues of limbs.

To date, thanks to advanced imaging and microscopy, much is known about the structural organisation of these cilia and flagella, yet their *in vivo* behaviour and coordination remains unclear. Defects in cilia and flagella have severe consequences for primitive algae and humans alike. Ciliary function can be impaired either directly due to structural mutation – for instance in patients suffering from PCD (primary ciliary dyskinesia), or indirectly due to build-up of extra-sticky mucus – as in cystic fibrosis. It is a remarkable feat of evolution (and testament to the success of this organelle) that mutations pertaining to mammalian cilia also affect proteins with close homologs in the flagella of green algae, most notably the model organism *C. reinhardtii* (Figure 1a). For the purposes of laboratory research we hope to gain invaluable insight by studying these ancestral flagellates, rather than their more advanced human counterparts.

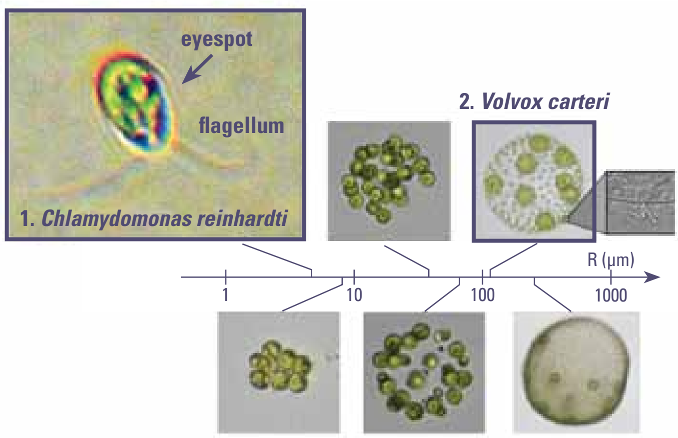

Figure 1a: A lineage of flagellate green algae, with species arranged in order of increasing cell size (and complexity). [Figure adapted from C. Solari *et al*, J.R.Soc.Interface 8, 1409–1417, 2011] Scale: 1μm = 10^{-6} m.

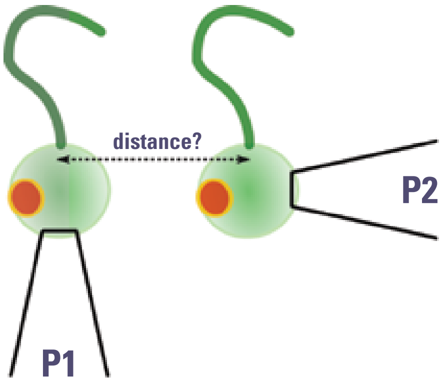

Figure 1b: By varying the distances of separation between pairs of somatic cells held on micropipettes (P1,2), we can study the role of hydrodynamics in coupling the beating of nearby flagella. [Brumley, Wan, Polin, & Goldstein, eLife, **3**, e02750 (2014)]

The aptly named *Volvox* – latin for 'fierce roller' – is a spherical alga (Figure 1a) which swims using thousands of flagella (think of a hairy ball). Its relatively large size renders this spectacular rolling motion visible to the naked eye. Two cell types are present in adult colonies: large germ cells in the interior, and adorning the surface are small, flagella-bearing somatic cells. Just like the arrays of cilia found in your lungs, these flagella produce waves of propulsion akin to metachronal waves in a stadium.

Herein lies the intrigue: how do you row your flagellum in sync with your neighbour? For decades, researchers have speculated that fluid dynamics lies at the heart of the phenomenon of 'coxless rowing' by cilia and flagella, and likewise the tendency for nearby spermatozoa to aggregate and swim off together. Indeed, that a beating flagellum might set up a local disturbance in the fluid which would in turn perturb the beating of a nearby flagellum seems like a tangible explanation. Furthermore, this effect would surely decay with increasing distance of separation between the two flagella. While we may be able to test this hypothesis using theoretical modelling or computer simulations, is there some way of controlling interflagellar spacing in a real-life experiment?

In the lab of Professor Ray Goldstein over at DAMTP (Centre for Mathematical Sciences), we have developed a framework for manipulating single micron-sized cells on micropipettes (hollow glass rods) so they can be readily visualised under the microscope. To obtain flagella-bearing cells, we mechanically sheared spheroids of *Volvox* to release the somatic cells, and captured these on separate micropipettes (Figure 1b). The relative distance between pairs of flagella can now be precisely controlled. Each flagellum operates like a metronome with its own intrinsic (or special) beat frequency which varies from one cell to another, analogous to the spread in the distribution of heights across the human population.

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For very small spacings, these pairs of tiny oars could overcome their difference in frequency and beat in unison – or synchrony, exactly as we had predicted; for larger spacings however, this synchrony is rapidly degraded, as the hydrodynamic

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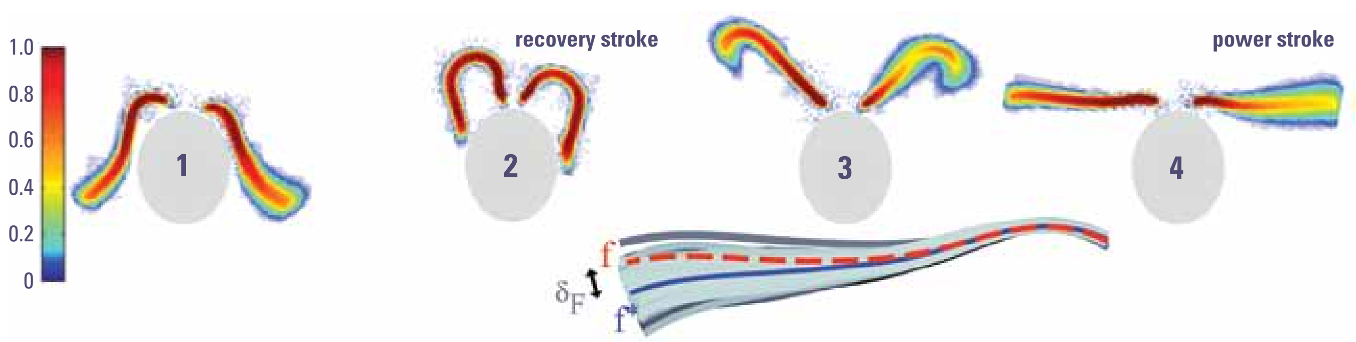

Figure 2: How *Chlamydomonas* choreographs its artful breaststroke: comparing the noisy waveforms produced by the flagella during different phases of its beat cycle. [Figure adapted from Wan & Goldstein, Phys. Rev. Lett. 113, 238103 (2014)]

coupling between them becomes far too weak. In quantitative terms, the phase difference – a measure of the difference between oscillators – grows over time without bound once the flagella are moved too far apart. Since the native spacings between flagella in *Volvox* are rarely more than one flagellum length, we must conclude that flagellar coordination in this alga can indeed arise spontaneously from hydrodynamic interactions alone!

Just how susceptible are flagella to deformations, by the action of the fluid or otherwise? To address this question let us take a closer look at *Chlamydomonas* (Figure 1a), which swims a characteristic breaststroke (Figure 2). Immobilising single cells on micropipettes, we can digitally track the locations of their flagella over tens of thousands of flagellar beats… When observed at equivalent phases of its nominally periodic beat cycle, each flagellum traces out curves which do not precisely overlap, even in the absence of obvious perturbations. Instead, the dynamics are inherently noisy, so there is some spread about an average shape. The degree of spread can then be assigned a numerical value by defining a notion of distance between curves, giving rise to a practical measure of flagellar waveform noise.

So why do fluctuations in flagellar beating persist in the absence of obvious perturbations? Harken back to the field of cardiology, where we find a well-known but nonetheless surprising result: the heartbeat of a healthy human individual at rest is far from perfectly rhythmic but instead displays rich, correlated dynamics, including sustained periods where beating occurs at above (or below) the average frequency. These behavioural signatures are very different in patients suffering from heart disease, or in subjects experiencing heavy physical stress.

The flagellum, as it turns out, is remarkably similar! We measure correlations in beating frequency lasting hundreds or even thousands of consecutive beats in healthy cells under normal conditions; yet when conditions become unfavourable or when cells are physiologically stressed, these slow frequency modulations are replaced by erratic, large amplitude fluctuations (Figure 3).

In greater detail than ever before, we can now resolve the spatial and temporal dynamics down to the sensitivity of a single cilium or flagellum; such methodological advances are crucial for deciphering the basis of signalling and control in this highly complex biomolecular machine, and will continue to inspire the next phase of our research.

For instance, we can pose the following inverse problem: given an observed flagellum shape can we derive the distribution of forces and bending moments that must be at work to produce it, and how must these be altered when the flagellum is perturbed – either mechanically, or as a consequence of mutagenesis? Or, from an evolutionary perspective, how many microswimmers come to possess not one but several flagella? Moreover, what additional strategies must be in play in situations where hydrodynamic interactions between these flagella may hinder rather than facilitate coordination?

Ultimately, translational applications of this work are anticipated. In establishing the consequences of ciliary dysfunction and mutation for human health and disease, the prevailing trend is undoubtedly for a more quantitative approach to be adopted, in order to maximise gain of useful information from the available biological data. Any such endeavour must be by its very nature interdisciplinary, and would have to incorporate experimentation, data acquisition and analysis, as well as mathematical modelling.

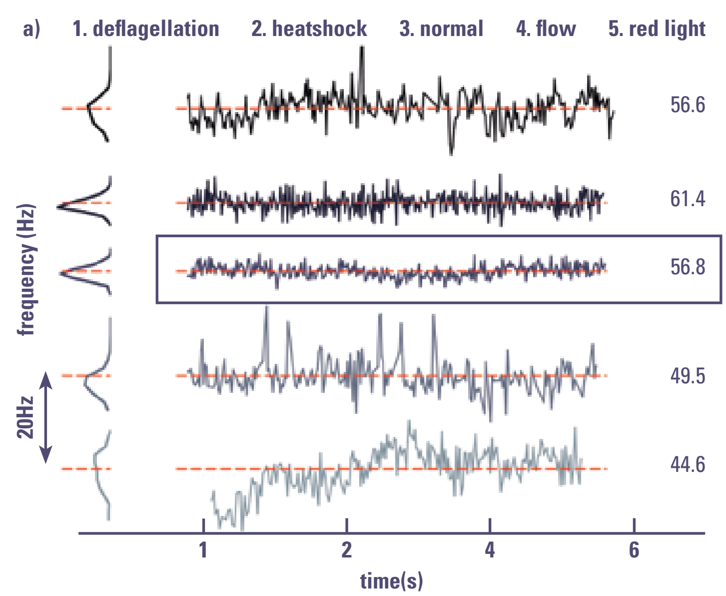

Figure 3. A *Chlamy's* 'heartbeat': timeseries of flagellar beat frequencies; the 'healthy' cell is highlighted.