

## Running tests on a miniature motor

Robert M. Macnab

Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06511 USA

When a team of automotive engineers tests out a new engine design, one of the characteristics they want to know about is how output torque varies with speed. Biophysicists who study biological motors are interested in this characteristic too.

A prime example is the bacterial flagellar motor. Bacteria swim using helical filaments, which are rotated by motors powered by protons (or in some cases sodium ions) descending the electrochemical potential gradient that exists across the cell membrane. The biophysicists face a couple of special difficulties in going about their task: One is that, since they did not build the motor, they have to try to decipher its structure and its mechanism as well as measure its characteristics. The other is that the motor is tiny,  $\sim 50$  nm in diameter, and so considerable ingenuity is needed to carry out the measurements. The paper by Iwazawa et al. in this issue exemplifies such ingenuity. These miniature motors have one advantage as a subject for analysis, namely, the fact that they operate in the laminar flow domain where drag is a strictly linear function of speed.

Many of the torque measurements have been made using a technique called tethering, which involves tying the cell down to glass by a single filament so that only the cell rotates. It has the advantage that the frictional geometry is fairly simple, and the cell body (unlike the 20-nm diameter filaments) is readily visible.

Berg and his colleagues have made extensive measurements of this kind, with the basic result that within experimental error torque was constant (i.e., if you double the viscosity, you halve the speed) over the measured range of speeds, up to  $\sim 10$  Hz (Manson et al., 1980). This is the result expected if the motor uses a fixed number of protons and is operating close to equilibrium, so that almost all of the proton's energy is being utilized against the external frictional load; or, to put it another way, the proton movements within the device are not rate-limiting. In a clever experiment involving flow of liquid past the rotating tethered cell, Meister and Berg (1987) extended these measurements to the limit of stall, and concluded that the stall and running torques were about the same.

The measurements just described were all in the high-load domain. Measurements have also been made in the low-load domain, using free-swimming cells and extracting the flagellar frequency from the power spectral density of scattered radiation. Here it was found that torque

dropped linearly with speed (Lowe et al., 1987), indicating that significant energy was being dissipated internally. The saturation speed that applies in the limit of negligible external load is  $\sim 100$  Hz, about an order of magnitude higher than in the tethered cell situation.

In order to explore intermediate ranges of motor speed, and also negative speeds (where the flagellum would be functioning as a generator rather than a motor), interest has developed in being able to apply external torque to the motor. The flow experiment mentioned above is a simple example, using imposed hydrodynamic force, but what is really needed for further analysis is the application of a rotating force field.

In the first example of such an experiment (Block et al., 1989), a tethered cell was subjected to optical trapping near the focal point of a laser beam; these "optical tweezers" were then used to drive the cell's rotation. However, this approach has primarily been used to measure the structural properties of the flagellum (specifically the torsional rigidity of the flagellar filament and hook) rather than the energetic properties of the motor.

In unpublished work, Berg and colleagues have used rotating magnetic fields to drive cells to which tiny magnetite needles have been artificially attached (a variant of a remarkable phenomenon encountered in certain naturally magnetic bacteria); while these workers succeeded in driving the cells, they were not able to make the necessary estimates of torque because of uncertainties in the orientation and strength of the attached magnetic dipoles.

The approach taken both by Iwazawa et al. (1993), and independently by Washizu et al. (1993), is to supply a rotating electrical field. There are major differences in how the two groups supply and use this field. Iwazawa et al. take advantage of cell electrophoresis (the cell carries net charge) and electro-osmosis (the medium flows in an electric field), and are able to entrain the cell at the field speed as long as the field is at or above a threshold amplitude; below that amplitude the cell rotates at its natural speed, because the phase relationship is broken and the mean torque is zero. Washizu et al., in contrast, use extremely high speed fields (500 kHz) to induce an electric dipole in the rotating cell; because of the finite relaxation rate, the orientation of the induced dipole always lags the field dipole and so the cell experiences torque.

Over the entire range they explored, from about  $-20$  Hz through  $0$  Hz (the "generator" range), through about  $10$  Hz (the "natural motor" range), and to about  $50$  Hz

(the "driven motor" range), Iwazawa et al. find within experimental error a linear relationship between the speed and the threshold amplitude, implying a linear speed-torque relationship. This is similar to the result reached for the high-speed range using free-swimming cells.

Various theoretical models have been developed for the motor mechanism (e.g., Iwazawa et al., 1993; Luger, 1988; Meister et al., 1989), which predict that torque should be a decreasing function of speed. The predictions, however, all deviate from a simple linear relationship and vary in the nature and extent of the deviation. With further refinement in the experimental measurements, it may be possible to discriminate between the various treatments and decide which of them most closely represents reality.

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