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Citation	Yuan, Junying, and Howard Curtis Berg. 2009. Following the behavior of the flagellar rotary motor near zero load. <i>Experimental Mechanics</i> , published online 09 September 2009. http://www.springerlink.com/content/5np0175062378620/
Published Version	doi:10.1007/s11340-009-9290-1
Accessed	February 18, 2015 5:36:43 PM EST
Citable Link	http://nrs.harvard.edu/urn-3:HUL.InstRepos:4729513
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Following the Behavior of the Flagellar Rotary Motor Near Zero Load

J. Yuan • H.C. Berg

Abstract At room temperature at stall, the flagellar motor of the bacterium *Escherichia coli* exerts a torque of ~1300 pN nm. At zero external load, it spins ~330 Hz. Techniques for studying the motor near zero load are novel and are summarized here.

Keywords Bacterial motility • Light scattering • Nano-gold

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Introduction

Many bacteria are propelled by the rotation of helical filaments that extend out into the external medium (in common bacteria) or run under the outer cell membrane (in spirochetes). Each filament is driven at its base by a reversible rotary motor embedded in the cell wall, constructed from about 20 different kinds of parts, about 50 nm in diameter, and powered by an ion flux (either protons or sodium ions); for a quick guide, see Berg [1]. The torque-speed relationship is distinctive: torque is maximum at stall (~ 1300 pN nm in *Escherichia coli* at room temperature), it falls $\sim 10\%$ between 0 and ~ 160 Hz, and then it drops rapidly, reaching 0 at ~ 330 Hz [2]. At speeds between 0 and 160 Hz, rates of ion translocation or movement of internal parts do not matter (torque is independent of temperature); at speeds above 160 Hz, these rates do matter (torque increases with temperature). So it is of interest to study the motor at small loads, where it runs far from thermal equilibrium, with an output that is sensitive to the kinetics of motion of internal parts.

At high loads and low speeds, one can study the motor by tethering a cell to glass by a single flagellar filament and watching the cell body pinwheel; see [3]. The body is cylindrical in shape with hemispherical end caps, about $1 \mu\text{m}$ in diameter by 2 to $3 \mu\text{m}$ long. Alternatively, one can break off most of the filaments by viscous shear and attach a latex bead to one of the flagellar stubs; see [4]. The smallest latex beads that have proved practical are $\sim 0.36 \mu\text{m}$ in diameter, enabling a motor speed of ~ 230 Hz. To work at lower loads, the most promising method appeared to be the gold nano-rod technique used by Wayne Frasch to study rotation of the F_1 ATPase [5]. Such rods scatter light strongly

in the red when the plane of polarization of the light is parallel to the long axis of the rod and less strongly in the green when the plane of polarization is normal to the axis of the rod. After synthesizing such rods and learning how to link them to flagella, we realized that it was much easier just to buy commercially-made gold spheres and watch them wobble by laser light scattering. This has allowed us to study the motor at loads orders of magnitude smaller than before. We used genetics to remove the flagellar filaments and antibodies to link the spheres to the flexible coupling at the filament's base (the proximal hook, a cylindrical structure ~20 nm in diameter by ~55 nm long) [6]. Methods for doing this and for monitoring both speed and direction of rotation are reviewed here.

Methods and Results

Labeling Hooks with Gold Spheres. Spheres were conjugated with anti-rabbit IgG, which allowed them to label anything coated with rabbit IgG antibody. We followed a method adapted from Liao et al. [7]: anti-rabbit IgG (R5506, Sigma) was activated with succinimidyl 6-[3-(2-pyridyldithio)-propionamido]hexanoate (LC-SPDP; 21651, Pierce) following the instructions from the manufacturer; 2.5 μ l of the final solution was added to 500 μ l of a suspension of colloidal gold (diameter 60 to 200 nm; British Biocell International), and the mixture was incubated at 23°C for 2 h; then 10 μ l of 1 mM O-[2-(3-mercaptopropionylamino)ethyl]-O'-methylpolyethylene glycol 5000 (mPEG-SH 5000; 11124, Fluka) was added, and the mixture was incubated at 23°C overnight. Spheres activated in this way were linked to hooks treated with rabbit anti-FlgE antibody, as described in [6].

Laser Scattering Microscopy. Our original idea was to excite gold spheres with laser light by total internal reflection, using an apparatus developed for visualization of type IV pili [8]. In this device, a laser beam is directed from below to a quartz microscope slide via a 60° prism (the slide mated to the prism with glycerol), and the light is totally reflected at the quartz-water interface. For work with gold, we used a 655-nm diode laser (Sanyo DL5147-042, run at ~8 mW with a DLC500 controller, Thorlabs), with its plane of polarization horizontal. The scattered light was viewed from above in an upright microscope with a Nikon Plan 40/0.65 BM objective. But it proved more convenient to use a flow cell [9] with a quartz bottom window instead of a quartz slide, and more convenient still to attach the bacteria to a glass coverslip that served as the top window of the flow cell. So we simply increased the angle of the laser beam so that the light passed through the quartz-water interface but was totally internally reflected at the glass-air interface, thus converting the microscope from total internal reflection to dark field. In the embodiment of this apparatus used to measure motor speed [6] the scattered light was focused onto a 0.2-mm-diameter pinhole in front of a photomultiplier tube (R7400U-20, Hamamatsu), yielding signals of the sort shown in Fig. 1. In a newer embodiment used to measure motor direction as well as speed [10], the light was focused, via a 50/50 beam splitter, onto two 3×1 mm precision slits (NT39-908, Edmund Optics) mounted at right angles to one another in front of two photomultiplier tubes (as above). When the center of rotation of the image of a gold sphere is aligned at one of the corners of the intersection of the slits, the phases of the signals from the two photomultiplier tubes

differ by +90 or -90 degrees, depending upon the direction of rotation of the motor, yielding signals of the sort shown in Fig. 2.

Discussion and Conclusions

Gold spheres on hooks of cells attached to glass – we use polylysine as glue – are not disturbed by bulk flow, so the preparations can be perfused with oxygenated motility medium, required to maintain protonmotive force (energized cell membranes). One can look at a single cell for hours; the sphere remains rigidly attached to the hook and there is no bleaching. So the method is ideally suited for experiments that require acquisition of switching data over long periods of time. The load can be increased somewhat by the use of larger spheres and/or the addition of the viscous agent Ficoll [10].

Acknowledgements This work was supported by National Institutes of Health Grant AI016478.

Figure Legends

Fig. 1. Measuring speed. The output signal from the photomultiplier tube oscillates as the image of a 60-nm gold sphere moves further on or off the pinhole. This trace shows the sudden onset of rotation of a motor with defective force-generating elements, resurrected by insertion of wild-type components; see [6].

Fig. 2. Measuring direction as well as speed. (A) the signal from the x photomultiplier tube shown as a function of time. The signal from the y photomultiplier tube (not shown) is similar, except for a 90° shift in phase. (B) the x, y signals shown in quadrature. When the motor changes direction, the circular trace changes direction, but this is not apparent in the figure.

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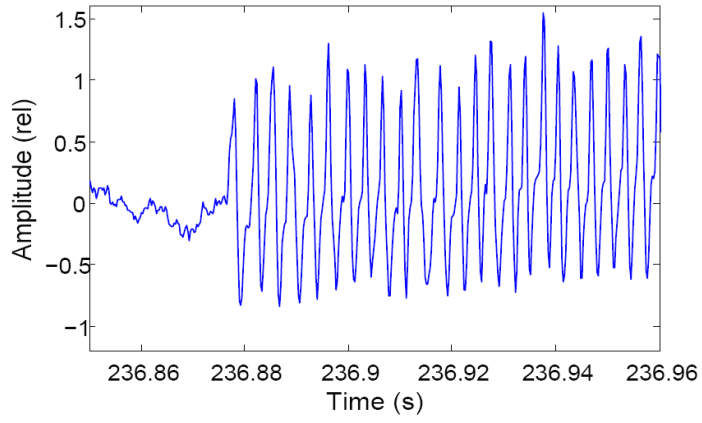


Fig. 1

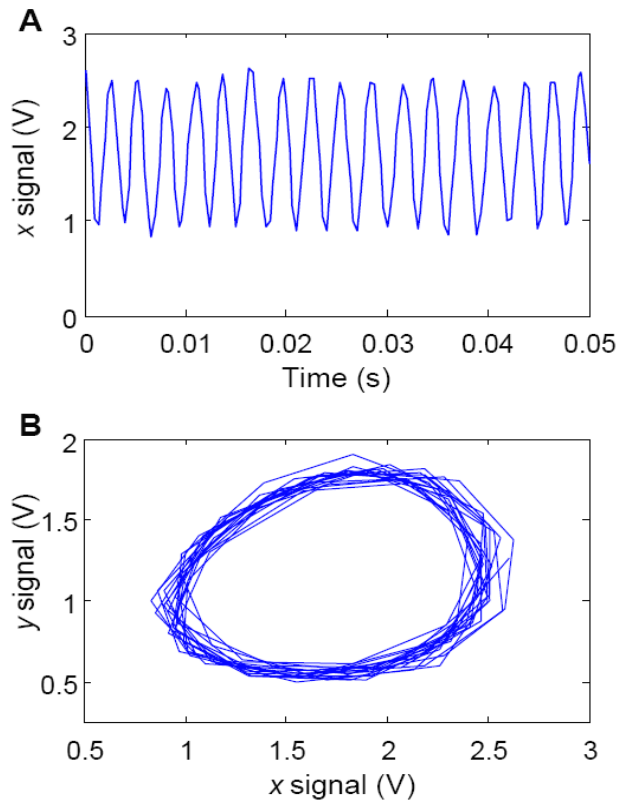


Fig. 2