Natural nanoscale machines

Modern imaging technologies have enabled novel single-cell studies, which are helping Professor Simon Rainville to gain in-depth knowledge about a fascinating biological nanomotor

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Could you begin by sharing the focal aims and objectives of your latest investigation?

Our research programme aims to understand how some biological motors work. We focus our research on an extraordinary biological machine; the bacterial flagellar motor. We are developing a unique approach to investigate the flagellar motor of *Escherichia coli* bacteria *in vitro*. We are also investigating new probes (using nanoparticles) and experimental techniques to visualise and precisely measure the motion of rotary biological motors. Using these tools we would like to answer questions, such as how the motor works at the molecular level and how this motor can rotate at over 20,000 revolutions per minute and then suddenly reverse its direction of rotation. This is a highly interdisciplinary field of research, combining Physics and Optics with Microbiology, Genetics and Chemistry.

Can you outline the ways in which your assay is unique?

Unlike other molecular motors, the bacterial flagellar motor has not yet been studied *in vitro* (ie. outside of the living cell). This is because it is more complex and needs to be imbedded in a membrane to properly assemble and function. Our approach is to hold a single bacterium partly inserted inside the tip of a tiny glass tube. Using an extremely short pulse of laser light, we then punch a small hole in the membrane of the cell to gain access to its interior. This system allows us unprecedented control over the experimental parameters, so we can achieve a precise study of the motor's physical and chemical characteristics.

What benefits can be derived from studying one cell at a time?

Most microbiology experiments are performed by taking average measurements on populations of microorganisms. Even though extremely successful, this approach has some important limitations. For example, it washes out oscillatory phenomena, variations between individual cells, and the importance of noise and fluctuations that are often critical to understand the underlying biological processes. Single-cell studies, such as the work in our laboratory, have been enabled by modern imaging and biophotonics techniques and allow detailed investigation of the essential processes at work.

What makes the bacterial flagellar motor so fascinating?

The bacterial flagellar motor is a nanotechnological marvel. It is a reversible rotary engine less than 50 nm in diameter. It is the output of an extraordinary signalling pathway inside the cell called the chemotaxis network, which allows bacteria to sense and respond to changes in the concentration of various chemicals in their environments. This mechanism allows many types of bacteria to move and find places that are more favourable for their development. Motility is obviously a crucial aspect of living organisms and it also plays an important role in the virulence of some pathogens.

Why is this process of such interest to nanotechnology researchers?

The field of nanotechnology aspires, among other things, to create machines and devices on the scale below the micrometre. The physical constraints are, however, very different at that scale compared to the macroscopic world we are used to building things in. Learning about the self-assembled molecular motors that have been around in nature for billions of years can greatly inspire us when designing nanoscale devices. Also, with a complete understanding of these machines, we could control them to perform various tasks, such as to carry a payload to a specific target in the human body or in microfluidic devices. This is only one aspect of an exciting new field of research called nanobiotechnology.

How do you support physics students in their pursuit of knowledge?

I had the incredible privilege to be mentored by great scientists throughout my student years, and I do my best to pass on the same scientific rigour and passion I received from them to my own students. I also give my graduate students autonomy, so that they can own the results of their work. Being a research supervisor is a challenging task, but also one of the most rewarding aspects of my job. Teaching is another passion of mine, where I try to communicate my love for science to students. Unfortunately our standard classroom lectures are often counter-productive in that students do not learn very much from them. I am very interested in physics education research and I try to improve my teaching using new, evidence-based teaching methodologies such as collaborative and active learning and using conceptually-based activities.

Inner **workings**

Using innovative biophysical and laser technologies, scientists at **Laval University** are advancing an *in vitro* assay to enable precise studies of the bacterial flagellar motor

THE DISCIPLINE OF bionanotechnology is offering new opportunities to delve ever more deeply into the biological mechanisms and processes at the nanoscale. One system receiving particular attention in that field is the bacterial flagellum, a rare example of rotary engines in biology. The bacterial flagellar motor contains approximately 13 different kinds of proteins and provides an ideal system to study molecular complexes. Moreover, this motor is the output of the bacterial chemotaxis pathway, a simple biochemical network inside the cell that represents a model system for intracellular signalling. Interestingly, there are still some fundamental questions outstanding about how this machine works, particularly with regard to the generation of the torque driving the rotation. With their explorations into the rotary motor of the bacterial flagella, a group of researchers at the Laval University's Department of Physics, Engineering Physics and Optics in Canada are hoping to add to this growing body of knowledge.

CHALLENGES OF WORKING AT THE NANO-SCALE

This work is based at a laboratory within the Center for Optics, Photonics and Lasers (COPL), and is led by Professor Simon Rainville. The information they are gathering forms part of the building blocks needed to understand more about the role bacterial flagellum plays in the mobility of living organisms. The bacterial flagellar motor, which drives this mechanism, is a remarkable machine. It is minuscule (less than 50 nm in diameter), spins at high velocities and can change its direction of rotation in an instant. This complex grouping of proteins needs to be imbedded into the bacterial membrane in order to function effectively. This means the motor is very difficult to study *in vitro*: "Such a system is an old dream in the field, and the idea comes from Howard Berg (Harvard University) who is a pioneer in the study of bacterial motility," explains Rainville. "We have built upon a system developed by Berg and his student David Fung, which was first reported in the mid-1990s." There are many technical challenges associated with its realisation, but thanks to the efforts of many talented students, the group has been able to successfully demonstrate such a system. The patch-clamp technique, a commonly-used method to investigate single or multiple ion channels, was adapted for very small cells and combined with some of the latest laser micromachining tools.

The assay consists of a filamentous cell that has been squeezed into a purpose-built micropipette. Femtosecond laser pulses offer the group an incredibly precise tool to access the inside of the cell. Ultrashort pulses of laser light are focused on the bacterium and burn a microscopic hole through the membrane. The benefit of the femtosecond laser, which acts like a highly precise nanoscalpel, is that very little damage is done away from the hole. In particular, the fragile flagellar motors implanted in the membrane micrometres away from the laser are left undisturbed. Surprisingly, the hole punched in the membrane of the cell open for a long time (15 minutes or more), providing direct access to the inside of the cell.

TAKING CONTROL

That direct access to the inner portion of the cell is essentially the reason why the investigators go through all these troubles. It allows them to gain control over the experimental conditions influencing the flagellar motor (or any structure in the membrane). In particular, the researchers

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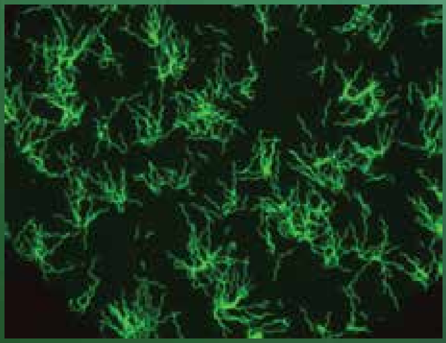
have had a direct influence over the rotation speed of the motor by applying an external voltage between the inside and the outside of the cell. They can stop and restart the motor at will, which represents a significant achievement in the field. The researchers are confident that this access to the inside to the cell will enable considerable insights into the physical and chemical characteristics of the bacteria flagella, for example allowing the identification of components that are essential for the switching mechanism. "There are many other aspects we will be able to address with this system, such as the steps in the rotation of a wild type motor and the dynamics of the torque generating units attached to the motor," Rainville highlights. To date, the team has focused their efforts on making the assay system more robust, and on developing reliable probes to precisely monitor the motor's rotation.

Whilst the focus of the project has predominantly been concerned with ensuring the *in vitro* assay is fully operational, the researchers have been able to undertake a number of small experiments on different features of the motor. For instance, they studied the unusual behaviour of a mutant bacteria identified by Dr R Harshey and whose filaments were detaching when swimming on agar. Through these tests it has been proven that this process was not, as previously hypothesised, influenced by the high proton-



E. COLI BACTERIA WITH FLUORESCENTLY LABELLED FLAGELLA

a number of different pathways, including presentations at national and international conferences. The first quantitative results should soon be published in a scientific journal, but a technical description of the method and preliminary observations were reported in *Physics in Canada* (the technical journal of the Canadian Association of Physicists) and the Proceedings of SPIE, the International Society for Optics and Photonics, in 2008. Thanks to an important award from the Canada Foundation for Innovation (CFI), the group is currently setting up a world-class research infrastructure including the latest technologies for high-resolution imaging, detection and manipulation of biological systems.

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motive force within the cell. The team is now keen to continue building the quantitative data gathered from their operational *in vitro* studies. Because of its originality and versatility, their bacterial *in vitro* assay shows great promise to become valuable technologies to investigate not only the motility and chemotaxis apparatus of *E. coli* and other bacteria, but also many other biological questions, especially other membrane-bound systems that are difficult or impossible to isolate otherwise.

SUPPORT

This research has been made possible through funding from the Natural Sciences and Engineering Research Council of Canada (NSERC), the Research Fund of Quebec – Nature and Technology (FQRNT). The majority of the award has been used to support the graduate students who form the backbone of the laboratory and work. In addition, the funding is used to help disseminate the results through

The facilities and knowledge available at Laval University have played a fundamental role in supporting this important biophotonics research. The COPL research group alone involves over 30 professors from across Quebec and has built a global reputation as one of the preeminent institutions for optics and photonics training and research. With a state-of-the-art Pavillon d'optique-photonique that is valued at over US $40 million, it certainly offers academics and students alike the opportunity to undertake pioneering optics research. "We also have the chance to interact with the nearby Neurophotonic Center, an interdisciplinary facility equipped with advanced and custom-designed optical systems for cellular and *in vivo* imaging of neuronal signalling and communication," notes Rainville. By working closely with expert physicists, chemists, microbiologists and neuroscientists, the Laboratory researchers are able to advance innovative transdisciplinary methods and will hopefully break new ground in our understanding of complex biological phenomena.

INTELLIGENCE

BIOPHYSICAL STUDIES OF THE BACTERIAL FLAGELLAR MOTOR

OBJECTIVES

- To develop novel technologies like an *in vitro* system that will enable detailed investigations of the flagellar motor that many bacteria (such as *E. coli*) use to swim in their environment
- To understand at the molecular scale how this fascinating motor functions; for example, how it can rotate at over 20,000 rpm and how it is able to suddenly reverse its direction of rotation
- To study the biophysics of bacterial motility more generally

KEY COLLABORATORS

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