JAPAN NANONET BULLETIN - 11th Issue - February 5, 2004

NANONET INTERVIEW

PDF Version (7.03MB)

Keiichi NAMBA
Professor, Graduate School of Frontier Biosciences, Osaka University

Revealing the mystery of the bacterial flagellum — A self-assembling nanomachine with fine switching capability —

(Issued in Japanese: March 25, 2003)

Keiichi NAMBA

Professor, Graduate School of Frontier Biosciences, Osaka University

1974 Bachelor of Engineering, Biophysical Engineering, Faculty of Engineering Science, Osaka University, Japan

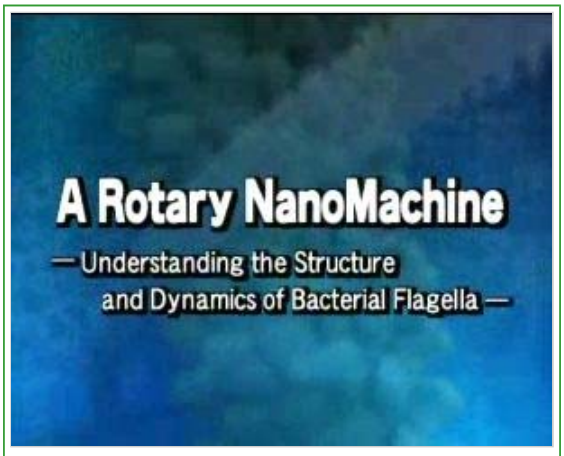
1980 Ph.D., Graduate School of Engineering Science, Osaka University, Japan
Postdoctral Fellow, the Japan Society for Promotion of Science, Graduate School of Engineering Science, Osaka University, Japan

1981 Research Associate, Brandeis University, Massachusetts, USA

1984	Research Associate, Vanderbilt University, Tennessee, USA
1985	Research Instructor, Vanderbilt University, Tennessee, USA
1986	Senior Research Associate, Brandeis University, Massachusetts, USA Group leader, Hotani Molecular Dynamic Assembly Project, ERATO, Japan Science and Technology Corporation
1992	Research Director, International Institute for Advanced Research, Matsushita Electric Industrial Co., Ltd., Japan
1997	Project Director (joint appointment), Protonic NanoMachine Project, ERATO, Japan Science and Technology Corporation, Japan
1999	Research Director, Advanced Technology Research Laboratory, Matsushita Electric Industrial Co., Ltd., Japan
2001-	Visiting Senior Researcher (joint appointment), Japan Synchrotron Radiation Research Institute, Japan
2002-	Professor, Graduate School of Frontier Biosciences, Osaka University, Japan
2002-	Project Director (joint appointment), Dynamic NanoMachine Project, ICORP, Japan Science and Technology Corporation, Japan

Award

2001	19th Osaka Science Award
2002	Outstanding Performance Award of Visual Science Festa 2002



VIDEO: A Rotary NanoMachine

— Understanding the Structure and Dynamics of Bacterial Flagella —

Play (36MB 34 Min.)

Windows Media Player is required to view this video.

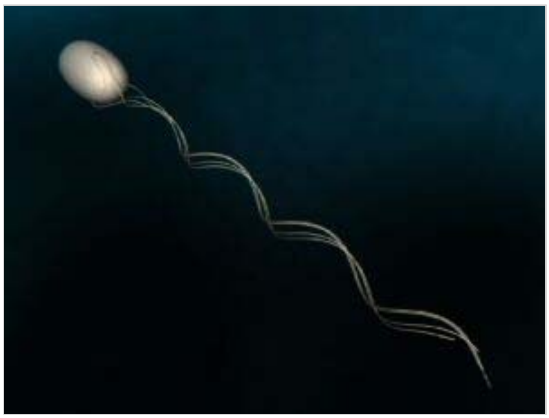


Fig. 1 Large Image

Bacterial cell in the straight swimming mode

Several flagella with a left-handed helical shape form a bundle behind the cell body and their synchronous rotation at 200 to 300 Hz generate the thrust.

Fig. 2 Large Image

Enlarged view of a bacterial cell in the straight swimming mode

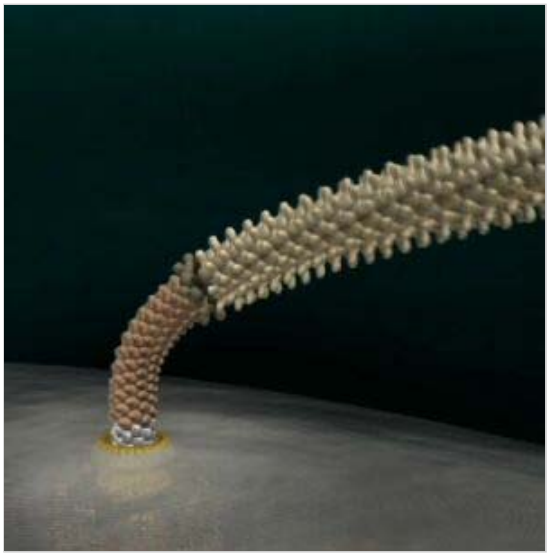


Fig. 3 Large Image

Enlarged view of the base of the flagellum

The rotary motor, shown semitransparently, is embedded in the cell membrane at the base of a helical filament. A short segment (about 55 nm) that connects the motor and the helical propeller is called “hook” and works as a universal joint.

Nature created a rotary motor with a diameter of 30 nm. Motility of bacteria, such as *Salmonella and E. coli* with a body size of 1 ~ 2 microns, is driven by rapid rotation of a helical propeller by such a tiny little motor at its base. This organelle is called the flagellum, made of a rotary motor and a thin helical filament that grows up to about 15 microns. It rotates at around 20,000 rpm, at energy consumption of only around 10^{-16} W and with energy conversion efficiency close to 100%. Prof. Namba’s research group is going to reveal the mechanism of this highly efficient flagellar motor that is far beyond the capabilities of artificial motors.

The flagellum is made by self-assembly of about 25 different proteins. The rotor ring made of protein FliF is the first to assemble in the cytoplasmic membrane. Then, other protein molecules attach to the ring one after another from the base to the tip to construct the motor structure. After the motor has been formed, the flagellar filament, which functions as a helical propeller, is assembled. Precise recognition of the template structure by component proteins allows this highly ordered self-assembly process to proceed without error. The flagellar filament is made of 20,000 to 30,000 copies of flagellin polymerized into a helical tube structure. Flagellin molecules are transported through a long narrow central channel of the flagellum from the cell interior to the distal end of the flagellum, where they self-assemble in a helical manner by the help of a cap complex. The cap is pentameric complex made of HAP2 and has a pentagonal plate and five leg domains, whose flexible stepping movements accompanied by rotation of the whole cap is the key mechanism to promote the efficient self-assembly of flagellin molecules by preparing just one binding site of flagellin at a time and guiding the binding.

Even though the filament is a polymer of chemically identical molecules, it conforms a supercoiled structure. By using electron cryomicroscopy and X-ray fiber diffraction, Prof.

Namba's group has discovered that the flagellar filament consists of 11 strands of protofilaments with two slightly different conformations, named L and R types. The repeat distance observed in the structure of the L-type protofilament is 5.27 nm, while it is 5.19 nm in the R-type, the difference being only 0.08 nm. The mixture of protofilaments with the different lengths produces the helical tube structure of the filament.

Bacterial cells swim actively by rotating a bundle of flagella. The motor switches its direction every few seconds to change the swimming direction of the cells for bacteria to seek better environments. Reversal of the motor rotation causes a structural change of the flagellar filament from the left-handed to the right-handed helical form. This makes the flagellar bundle fall apart, propelling force imbalanced, leading to changes of the swimming direction. The switch that triggers this change in the helical form of the filament has been found in the atomic structure of flagellin obtained by X-ray crystallographic analysis. When the twisting force produced by quick reversal of the motor rotation is transmitted to the protofilaments, part of flagellin undergoes a slight change in its conformation, thereby making a few of the 11 protofilament strands transform from the L-type into the R-type. As a result, normally left-handed flagellar filament turns into right-handed helical forms. Prof. Namba's group tried to understand the switching mechanism responsible for these structural changes. To analyze the structure in atomic detail by X-ray crystallography, flagellin had to be crystallized. However, its strong tendency of polymerization made the crystallization difficult. It took ten years for them to finally crystallize flagellin and analyze the structure to find out the switch mechanism, for which a super brilliant X-ray beam from SPring-8 beamlines was essential.

Prof. Namba first saw an electron micrograph of the bacterial flagellum and its motor when he was a graduate student. He was surprised to see such complex and sophisticated structure exist in living organisms. It impressed him deep enough to switch his research from muscle to flagella after a while. "Looking at the shape of the flagellar basal body, it is obviously designed to rotate. Looking at a picture of the flagellar motor on the wall every day, I feel up towards revealing the mystery by any means." The design concepts of protein molecules to realize various functional mechanisms by their three-dimensional architecture are quite different from those we design by our engineering technique with bulk materials. Folding of single polymer chain into some three-dimensional structures gives a huge amount of freedom and flexibility in both function and structure. Individual atoms are used as functional parts, and this is the essential feature that makes biological macromolecules distinct from artificial machines at present. The design concepts have to be well understood and learned for future nanotechnology applications. So far, for the flagellar motor, the deeper our insights get into the mechanism, the deeper the mystery becomes. Now the mystery of conformational switching of the filament has been solved, and in terms of the number of protein molecules, the filament makes up 99% of the entire flagellum, it does not mean 99% of the mystery is solved. It is the motor mechanism that is even more difficult to understand.

When Prof. Namba's group attached a 40 nm fluorescence bead to the flagellar motor and observed the motor rotation, the group was surprised to see large and rapid fluctuations of the rotation speed. The key to revealing the mystery of the motor must be hidden behind the thermal fluctuation of the protein structure, which is still so far from understanding. "The atoms constituting proteins do fluctuate but the average positions of individual atoms are very precisely

determined with an accuracy of sub-angstrom level. That is why individual proteins can properly identify partner molecules to bind and get assembled into the higher order structures of living organisms. The fluctuations of protein structure, that's what makes living organisms function in such sophisticated and well regulated ways. I am willing to dedicate my entire life to the hard work unveiling the mysterious world of protein structure and function."

(Interviewer: Kuniko Ishiguro, Cosmopia Inc.)

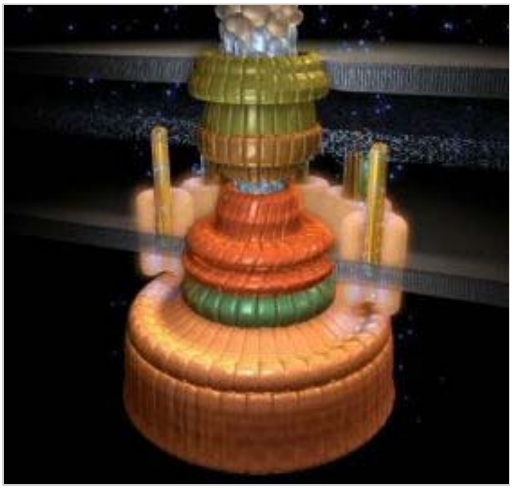


Fig. 4 Large Image

Schematic diagram of the flagellar motor

The space at top and bottom is the cell exterior and interior, respectively. The flagellar motor is a macromolecular assembly made of approximately 20 different proteins. It spans across three layers of membranes, namely, the cytoplasmic membrane, the peptidoglycan layer, and the outer membrane, from the bottom to the top. It consists of various components, such as a rotor, stators, a drive shaft, a bushing, a rotation-switch regulator, and so on.

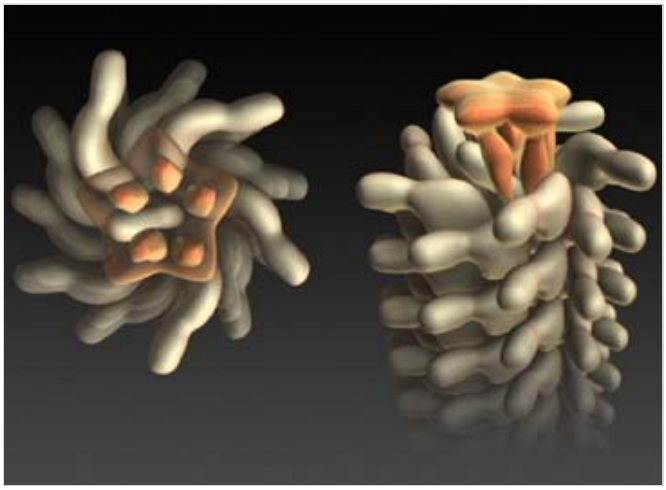


Fig. 5 Large Image

Rotary cap mechanism to promote efficient self-assembly of flagellin

Flagellin molecules synthesized in the cytoplasm are transported to the distal end of the filament through its long narrow central channel. These molecules bind one after another to the distal end, where the flagellar cap, by its binding to the filament over symmetry mismatch, prepares just one flagellin binding site at a time and rotates upon flagellin binding to prepare the next binding site. The rotary cap movements look as though it is climbing up the helical stairs step by step.

Fig. 6 Large Image

Partial atomic model of the flagellar filament in the end on view

This filament model is constructed with the atomic model of the F41 fragment of flagellin obtained by clipping off 52 and 44 amino acid residues from each terminal region of flagellin comprised of 494 residues. The diameter is 23 nm. The central channel appears large because the inner core domain formed by both terminal regions is missing in this model. The actual internal diameter of the channel is only about 2 nm.

Fig. 7 Large Image

Self-assembly process of the bacterial flagellum

The process goes from the top left to the bottom right corner. Once the FliF ring (in brown) has formed in the cytoplasmic membrane, other protein molecules are self-assembled on this structural base one after another in a well-defined order. All the axial component proteins of the flagellum are synthesized in the cytoplasm and transported by the type III flagellar protein export apparatus through the long narrow central channel to the distal end of growing structure, onto which they self-assemble. The assembly process requires three different types of caps at different stages, and these caps are always attached at the distal end of the growing structure to promote efficient self-assembly of component molecules.

| JNNB Top | Subscribe Now! | INTERVIEW | YOUNG RESEARCHERS | TEXT | Past Issues |

JNNB Search Search

| Home | Site Map | Contact Us | To Page Top |