Effects of Membrane Tension on Nanopropeller Driven Bacterial Motion

Roohi Gupta, Megha Sharma, and Aditya Mittal*

Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology, Hauz Khas, New Delhi 110016, India

Our present capabilities to build nanomachines are very limited compared to the elegance and efficiency of bio-nanomachines. The flagellar motor of bacteria is an example of a bionanomachine. It is a structured aggregate of proteins anchored in many bacterial cell membranes (formed mostly from phospholipids). While a large body of work characterizes various functional components of flagellar proteins, limited literature exists on the role of phospholipids of the membranes anchoring the protein. It is assumed that the membranes do not play any active role in the nano-propeller's functioning. However, it is relevant to question this assumption for several reasons. Firstly, the anchor for any machine on any scale is essential in terms of the work-load the machine can deliver. Secondly, it is now clear that localized protein-lipid interactions are essential for functioning of many transmembrane proteins. These interactions result in formation of "nano-domains" of specific lipid constituents around the protein providing the desired functionality. Thus, regardless of whether the bacterial membrane is primarily an anchor for flagellar proteins or specific lipid components of the membrane are actively participating in nano-propeller driven motion of bacteria, it is important to investigate the role of the membrane itself in working of this bionanomachine. Using video microscopy with a 33 ms resolution to monitor bacterial motion, we investigate effects of varying the membrane tension, by providing different osmotic environments, on the performance of the flagellar motor. Our data strongly demonstrate an active role of bacterial membranes in the nano-propeller driven bacterial motion. Our results point towards reconsidering performance of classical bionanomachines like bacterial flagellar motor and F1–F0 ATPase in view of the membranes in which they are packed in, in contrast to just the proteins by themselves.

Keywords: Bio-Nanomachine, Flagellar Motion, Motility, Osmolarity, Membrane Tension, Phospholipid Packing.

1. INTRODUCTION

A bio-nanomachine can be defined as the objects made up of multiple molecular components to perform a specific biological function within or outside a living cell. The flagellar motor system of bacteria1,2 is an example of a bio-nanomachine. It is a structured aggregate of proteins anchored in many bacterial cell membranes, which are formed mostly from phospholipids. It has been found that the flagellar protein works like a propeller allowing the bacterial cells to propel in search of a better living environment. It rotates at about 20,000 rpm with an energy consumption of only around $10^{-16}$ W and an energy conversion efficiency of nearly 100%.1

Figure 1 shows the various components of the prototypical flagellar motor of the bacterium *Escherichia coli* (referred to as *E. coli* subsequently in this paper). The bacterial nano-propeller (often referred to as the filament) is composed of the protein flagellin and is a hollow tube 20 nm thick. It is helical, i.e., has a helix structure, and has a sharp bend just outside the outer membrane called the hook which allows the helix to point directly away from the cell. A shaft runs between the hook and the basal body, passing through protein rings embedded with in the outer bacterial cell membrane. The bacterial flagellum is driven by a rotary engine composed of protein components located at the flagellum’s anchor point on the inner cell membrane. The engine is powered by proton motive forces, i.e., by the flow of protons (i.e., hydrogen ions) across the bacterial cell membrane due to a concentration gradient set up by the cellular metabolism.3

Other ionic gradients like those of Na$^+$ are also known to drive specific motors. The exact nature of the mechano-chemical coupling leading to transformation of concentration gradients to mechanical energy resulting in nano-propeller motion is still a very active area of
RESEARCH ARTICLE

Gupta et al.

Role of Membrane Tension in Flagellar Motion of E. coli

Fig. 1. Components of a flagellar motor in a typical gram negative bacteria. Note that the cartoon is drawn somewhat to scale and does not contain all molecular details but does highlight the “mechanical” description of the components of the nano-motor. The scale of components can be estimated by considering the “L” protein ring (shown in the outer membrane) whose diameter (when approximated as a cylinder) is about 20 nm.

It is important to note in Figure 1 that the flagellar nano-propeller is anchored in two membranes, i.e., the outer and the inner membranes, of the bacterial cell. These membranes are comprised mainly of phospholipids and act as separation barriers between different environments existing inside and outside the bacterial cell. While a large body of work is dedicated to characterizing various functional components of the flagellar protein, negligible literature exists on the role of phospholipids of the membranes anchoring the protein.

Now, simply from a mechanical point of view, the anchoring for any machine on any scale is essential in terms of the work-load the machine can deliver. From a structural engineering stand point, it is pertinent to know what kind of soil/earth characteristics exist, before a mechanical structure, especially one which needs to continuously move at a fixed place for the desired function, can be built in a particular location. For the bacterial nano-propeller system, this anchoring comes in the form of not one but two biological membranes. Therefore, the obvious question from a nanotechnological stand point is that, to envision construction of a synthetic nano-propeller system by learning from the existing highly efficient natural bacterial propeller system, is it important to consider the membrane environment in which one would like to anchor this nano-machine or not? To answer this question, the first approach has to be simply looking at the effects of phospholipid density in the bacterial cell membrane on the nano-propeller performance. Phospholipid density of biological membranes can be varied using different osmotic conditions and the nano-propeller performance can be studied in terms of the actual bacterial motion. Thus, we ask, whether there exists an optimal osmotic condition, in turn representing optimal phospholipid packing density, at which the bacterial motility is maximum or not, which in turn reflects on the maximal activity of the bacterial nano-propeller. Figure 2 shows the presumed effects of various osmotic conditions on the bacterial membranes. Suppose Figure 2(A) shows the bacterial phospholipids density at iso-osmolar conditions. Then Figure 2(B) shows the effect of a hypotonic solution on the phospholipids density, which is decreased, and Figure 2(C) shows the effect of a hypertonic solution on the phospholipids density, which is increased. Note that Figure 2 is a somewhat over-simplified diagrammatic representation of effects of different extracellular osmolarities on the bacterial cell membranes and is not to scale. In reality, the outer and inner monolayer phospholipid densities may be slightly different, as well as the areas per head groups and lipid tails may vary in response to osmotic stress. However, the overall effects of different extracellular osmolarities on bacterial cell membranes is well reflected by Figure 2.

Another important factor that needs to be addressed from a nano-technological perspective, to be able to assemble a synthetic nano-propeller inspired by the bacterial system, is whether a specific lipid–protein interaction is necessary for the nano-propeller to function. For a number of years, the “fluid-mosaic model” has been used to describe biological membranes, in which it is assumed...
that the membrane is a 2-dimensional fluid formed by phospholipids, that are randomly mixed, with a mosaic of proteins embedded in them. However, over the past decade the fluid-mosaic model has come under investigation in terms of its “fluid nature” not being of uniform characteristics. Rather, it has now been observed that biological membranes have nonuniform lipid and protein distributions with specific lipid–lipid, lipid–protein, and protein–protein interactions forming heterogeneities that give rise to localized domains within the membranes. These localized domains, often resulting in specific lipid–protein complexes, apparently vary in size from nm to μm scales. More importantly, these domains that are thought to spontaneously form due to the specific lipid–protein and lipid–lipid interactions have been implicated in a variety of physiological functions in eukaryotic cell biology. While existence of these domains has been well demonstrated with eukaryotic cell membranes, it is possible that they play an important role in bacterial membranes also. Considering this alternative, one would predict that varying the overall phospholipid density would not affect the localized packing environment (i.e., specific membrane lipids complexed with the protein) of a nano-domain constituting the bacterial nano-propeller and in that case the bacterial motion, representing the nano-propeller performance, would be insensitive to different osmotic conditions.

Therefore, in view of the above and considering that membranes are not considered to play any active role in the nano-propeller functioning, we decided to investigate the role of phospholipid density in bacterial motility, by varying osmotic conditions. Below, we present experimental evidence highlighting the role of bacterial membrane in the nano-propeller driven bacterial motion. We find that the biological nano-propeller performance is indeed sensitive to phospholipid density, as indicated by significant changes in motility characteristics resulting from varying osmotic conditions.

2. EXPERIMENTAL PROCEDURES

*E. coli* strain JM109 was used for this study. The strain JM109 was chosen since it has been found to be one of the most motile strains of *E. coli*. Nutrient broth, NaCl, Sucrose, and other miscellaneous chemical reagents, all of analytical grade, were obtained from Himedia, Qualigens, and Merck.

2.1. Methods

*E. coli* cells were grown in 100 ml nutrient broth in a 500 ml flask at 37 °C until the exponential phase of growth (6 hours). Bacteria were then rapidly pelleted and resuspended in sodium phosphate buffer solution (Osmolarity 300 mOsm) at room temperature. This was done to equilibrate all the bacterial samples towards the same osmotic conditions so that immediate osmotic changes experienced by the bacteria could be monitored. 250 μl drop of cell suspension (in buffer solution) was added to each 1 ml solution of water, buffer or media having different osmolarities. Different osmolality solutions were prepared by adding NaCl and sucrose in two different sets of experiments for solutions with each water, buffer, and culture medium. Note that while the osmolarities of water and buffer were exactly calculated, the media osmolarity was simply changed by adding different NaCl or sucrose since original medium osmolarity was not estimated. Thus, for the experiments with medium, the solutions were with increasing hypertonicity only. Immediately (within a few seconds) after addition of cell suspension to the solution with desired osmolarity, samples were transferred to slides, covered with the cover slip and digital videos of bacterial motion were recorded using Motic B1 upright microscope with digital camera acquiring the images at the rate of 30 frames per second (i.e., 33 ms time resolution) using the software Motic images Plus 2.0 ML with the 100X oil objective. Note that our motility recordings were done within 30–60 s of introducing the bacteria to the new osmotic conditions in order to observe the osmotic effects only, without giving a chance to the bacteria to respond biologically (e.g., trigger genetic responses) and adapt to the new conditions. Thus, once a sample was observed and recorded for about 10 s, it was discarded followed by the next experiment. Each experiment was done in duplicate. Average number of bacteria per sample (i.e., per field of view) were 31 with a range of 11 to 75 single bacterial cells in different experiments.

3. RESULTS

3.1. Assessing Nano-Propeller Performance Based on Bacterial Motility

Nano-propeller driven bacterial motility has been studied extensively as a part of a wide body of literature investigating chemotaxis (chemically induced signaling leading to movement in the direction of better environments) in bacteria. The motility of bacteria resulting from flagellar motor’s function in response to chemical signals has been parameterized in terms of runs, twiddles, straight paths, clockwise rotations, circular trajectories, “driving to the right;” and tumbles. Our assay for assessing the nano-propeller performance was to observe motility of bacteria considering all of the above parameterizations. Thus, in a given field of view under the microscope, we considered any of the above motions as flagella driven motion and counted the bacteria as motile. This allowed us to easily differentiate the nano-propeller driven motile bacterial cells from those that were simply drifting as a result of fluid movement on the slide or just dead cells floating randomly in the sample. Figure 3 shows 5 images at intervals of t = 0, 33, 66, 500, and
3.2. Effects of Osmotic Stress on the Nano-Propeller Performance Based on Bacterial Motility

Figure 4(A) shows the effect of osmolarity on flagella-driven bacterial motility in water. Circles show the data when NaCl was used to prepare different osmolar solutions and squares show the data when sucrose was used to prepare the different osmolar solutions. It is interesting to observe that in the cases of lowest osmolarities, $\sim 40\%$ of bacteria are motile (reflecting $\sim 40\%$ of active nano-propellers) instead of lysing under presumably hypotonic solutions. Our observations are supported by previously reported electron microscopy studies$^{21}$ in which the maintaining of the bacterial cell integrity is seen in spite of harsh hypotonic conditions due to the cell wall preventing the bursting of the cell. There are a couple of striking features in Figure 4(A). Firstly, the bacterial motility (measured as fraction of motile bacteria) dependence on osmolarity is non-monotonous. With the exception of the first point for NaCl solution, bacterial motility is highest around 0.9 Osmolar (osmoles/liter) for both the solutions. That clearly demonstrates that the nano-propeller driven bacterial motility is clearly operative maximally at a particular membrane tension, in turn phospholipids density, equivalent to $\sim 0.9$ Osmolar extracellular concentration. Subsequently higher extracellular osmolarities show a continuous decrease in the motility. In mechanical terms, looking from strictly a nano-machine perspective, beyond 0.9 Osmolar the membrane anchor around the propeller is just too tight for the rotary operation. In other words, the increase in phospholipids density due to increasing extracellular hypertonicity leads to restriction of the flagellar nano-propeller’s rotary motion.
Figure 4 shows the effect of osmolarity on flagella-driven bacterial motility in medium. Considering that we did not measure the medium osmolarity, the X-axis values would shift to higher values. Regardless of the absolute value of osmolarity, a non-monotonous dependence on osmolarity in medium is similar to that observed in the previous two cases. However, it must be noted that the highest nano-propeller driven motility is achieved in the medium at the lowest osmotic contributions of both NaCl and sucrose. This suggests clearly that culture media conditions provide the right physical and chemical conditions for the best nano-propeller performance. That said, subsequent to decrease in motility after slight increase in extracellular osmolarity, the motility increases again around 1–1.5 Osmolarity values. This particular observation requires further investigation and can be attributed to multiple reasons, including the impact of chemotaxis resulting from abundant chemical signals in the medium being much more than simply the effects of phospholipids density. Therefore, considering Figures 4(A), (B), and (C) collectively the following results are can be summarized:

1. Nano-propeller driven bacterial motility is dependent on the extracellular osmolarity in a non-monotonous manner. There is an optimal membrane tension or phospholipids density at which the flagellar motor operates maximally, regardless of the manner in which the extracellular osmolarity is affected (i.e., with sucrose or salt).
2. The maximal possible motility of bacteria is achieved in absence of media components (≈80% for salt/sugar for water or buffer system compared to less than 60% in presence of medium regardless of salt or sugar). This makes physiological sense due to chemotaxis being highest in absence of a uniform nutrient availability when culture medium is not used.

Looking at the data in Figure 4, it is useful to combine all the data for a given set of physical and chemical conditions (i.e., group all data for sucrose regardless of water or buffer), without the medium, in order to learn about the overall dependence bacterial motility on osmotically controlled membrane tension. Figure 5 shows the overall dependence of nano-propeller driven motility on the extracellular osmolarity for NaCl (Fig. 5(A)) and sucrose (Fig. 5(B)) regardless of water or buffer. The non-monotonous behavior, along with the optimal phospholipids density in the bacterial membrane (represented by extracellular osmolarity), are clearly observed. Figure 5 shows that in absence of accessibility to nutrients, flagellar motility of bacteria operates at membrane tensions/phospholipids densities equivalent to 0.5–1.0 extracellular osmolarity.
3.3. Possible Role of Solution Viscosity

While NaCl has been a very well documented osmotic agent to study the effects of external osmolarities directly on the physical parameters of E. coli, in case of sucrose, one possible factor influencing motility of bacteria can be increasing viscosity of the solution resulting from higher concentrations of sucrose. Viscosities of sucrose solutions have been well documented. Hirst and Cox developed a mathematical relationship that was developed for sucrose solutions in the range of 0–50% (w/v) concentrations:

\[ \text{Viscosity} = \frac{6581}{[(61.5 + \Theta) - (1 + 0.011\Theta)C]^2} \]

where \( \Theta \) is the temperature (°C) and \( C \) is the sucrose concentration (g/100 ml). Figure 5(C) shows calculated viscosities of the sucrose solutions used by us by taking the temperature \( \Theta = 30 \) °C. Note that for calculation purposes, the sucrose concentration was corrected for the experimental protocol, where 0.25 ml of bacterial cells equilibrated in buffer were suspended in a 1 ml solution of sucrose (see experimental procedures for details). It is clear that up to ~1 Osmolar the changes in viscosities, while increasing, are very small. Above 1 Osmolar, the calculated viscosities show a sharp rise. Swimming velocity of bacteria has been well studied as a function of solution viscosity. It has been very elegantly demonstrated for another flagellated bacteria, Pseudomonas aeruginosa, that inverse of viscosity and swimming velocity follow a somewhat simple mathematical relationship, with the velocity decreasing with increasing viscosity. On the other hand, certain flagellar properties of cricket sperm have been reported to be independent of viscosity of the external medium.

In our data, it is interesting to visually note from Figures 5(B) and (C) that in the regime of mild changes in viscosity, the non-monotonous relationship of % motility with osmolarity is already observed (Fig. 5(B), peak motility is at 0.88 Osmolar). Beyond 1 Osmolar, the sharp increase in viscosity is not supported by a very sharp decrease in % motility.

To quantify the effects of (inverse of) viscosity and osmolarity independently on % motility, we investigated the presence or lack of correlations between the former two parameters with the latter by calculating the correlation coefficients (p). The results are summarized in Table I. It is clearly seen that when considering the data of only 0–50% (w/v) sucrose solutions (in which viscosity is mathematically calculated from osmolarity), the viscosity is not correlated with % motility (p = 0.08). While it appears that even osmolarity is not highly correlated with % motility (p = -0.14), it is still about twice as correlated compared to viscosity. However, the apparently low correlation of osmolarity with % motility is due to the non-monotonous behavior observed by us, as seen by the p values for subsets of the data in Figure 5(B). From 0–0.88 Osmolar, the % motility increases with increasing osmolarity with a fairly strong correlation (p = 0.45). From 0.88–5.54 Osmolar, the % motility decreases with increasing osmolarity with a very strong correlation (p = -0.84). Combining all

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Correlation coefficient with % motility</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–50% Sucrose</td>
<td>1/Viscosity</td>
</tr>
<tr>
<td></td>
<td>solution</td>
</tr>
<tr>
<td></td>
<td>Osmolarity</td>
</tr>
<tr>
<td>0 – 0.88 Osmolar</td>
<td></td>
</tr>
<tr>
<td>(0.88 – 5.54 Osmolar)</td>
<td></td>
</tr>
<tr>
<td>~0 – 6 Osmolar</td>
<td></td>
</tr>
</tbody>
</table>

In the experimental protocol, where 0.25 ml of bacterial cells equilibrated in buffer were suspended in a 1 ml solution of sucrose (see experimental procedures for details). It is clear that up to ~1 Osmolar the changes in viscosities, while increasing, are very small. Above 1 Osmolar, the calculated viscosities show a sharp rise. Swimming velocity of bacteria has been well studied as a function of solution viscosity. It has been very elegantly demonstrated for another flagellated bacteria, Pseudomonas aeruginosa, that inverse of viscosity and swimming velocity follow a somewhat simple mathematical relationship, with the velocity decreasing with increasing viscosity. On the other hand, certain flagellar properties of cricket sperm have been reported to be independent of viscosity of the external medium.

In our data, it is interesting to visually note from Figures 5(B) and (C) that in the regime of mild changes in viscosity, the non-monotonous relationship of % motility with osmolarity is already observed (Fig. 5(B), peak motility is at 0.88 Osmolar). Beyond 1 Osmolar, the sharp increase in viscosity is not supported by a very sharp decrease in % motility.

To quantify the effects of (inverse of) viscosity and osmolarity independently on % motility, we investigated the presence or lack of correlations between the former two parameters with the latter by calculating the correlation coefficients (p). The results are summarized in Table I. It is clearly seen that when considering the data of only 0–50% (w/v) sucrose solutions (in which viscosity is mathematically calculated from osmolarity), the viscosity is not correlated with % motility (p = 0.08). While it appears that even osmolarity is not highly correlated with % motility (p = -0.14), it is still about twice as correlated compared to viscosity. However, the apparently low correlation of osmolarity with % motility is due to the non-monotonous behavior observed by us, as seen by the p values for subsets of the data in Figure 5(B). From 0–0.88 Osmolar, the % motility increases with increasing osmolarity with a fairly strong correlation (p = 0.45). From 0.88–5.54 Osmolar, the % motility decreases with increasing osmolarity with a very strong correlation (p = -0.84). Combining all
the data for osmolarity (~0–6 Osmolar) versus % motility, a strong correlation is observed between decreasing motility with increasing osmolarity ($p = -0.82$). So, summarizing the results shown in Table 1, it is clear that even though viscosity may be playing a role in decreasing the % motility, the non-monotonous behavior of the % motility certainly has a major contribution from osmolarity, independent of viscosity. We believe that this major contribution from osmolarity comes in form of modulation of membrane tension.

4. DISCUSSION

*E. coli* cells are considered to be osmotically unchallenged at an osmolarity of 0.24 Osmolar. However, we clearly show that in absence of the media components or excessive nutrients, bacterial motility and in turn the nano-propeller performance, is actually not the most efficient at this osmolarity. In fact, the optimal extracellular osmolarity for the flagellar motor is 0.5–1.0 Osmolar. Therefore, regardless of whether NaCl or sucrose is used, the nano-propeller performance is enhanced on tightening the membrane anchor around it (i.e., increasing the phospholipids density) by putting a hypertonic solution outside the cells. Figure 6 shows a cartoon representation, not drawn to scale or molecular accuracy but representing the concept only, for the behavior observed in our data. Under hypertonic conditions, the propeller is “loose,” thereby decreasing the performance as shown in Figure 6(A). At the right membrane tension, the propeller packing is just right to be able to obtain the optimal performance as shown in Figure 6(B). Under hypertonic conditions, the propeller is too tightly packed, thereby again reducing the performance of the propeller and eventually bringing it to a stop as shown in Figure 6(C).

The model proposed in Figure 6 can be quantitatively analyzed by using the Laplace’s law for relating osmotic pressure and volume. Assuming the bacterial cell to be a cylinder, the tension in the bacterial membrane is given by $\tau = \Delta \Pi \cdot r$, where $\Delta \Pi$ is the osmotic pressure difference (given by $\Delta \Pi = R T \Delta Osm$, $\Delta Osm$ being the osmolality difference) across the membrane and $r$ is the radius of the cylindrical cell. While detailed calculations are beyond the scope of this work, a simpler set of calculations, based on measurements done on osmotic shrinkage or expansion of bacterial cells using very similar osmotic treatments as used by us, are given below to test the feasibility of our proposed membrane tension based mechanism in Figure 6. Let us assume the bacterial cell to be a cylinder of radius $r$ and length $h$. Let the outer membrane of the bacterial cell be of thickness $t$. Therefore, volume of the bilayer is easily found to be:

$$V_{\text{bilayer}} = \pi (r + t)^2 h - \pi r^2 h = \pi h (t^2 + 2rt)$$  \hspace{1cm} (1)

As per experimental observations let us assume an increase or decrease in the bacterial cell volume to be $\alpha$ times the original cell volume (i.e., $\alpha < 1$ when the cell shrinks due to external solution being hypertonic and $\alpha > 1$ when the cell swells due to the external solution being hypotonic). Therefore, by definition, new cell volume as a result of osmotic treatment is given by $V_{\text{osm}} = \alpha V_{\text{Original}}$. Assuming that changes in length of the bacterial cell are negligible (i.e., $h$ is the same), the new radius of the cylindrical cell is given by:

$$r_{\text{osm}} = r_{\text{Original}} (\alpha)^{1/2}$$  \hspace{1cm} (2)

Putting Eq. (2) in Eq. (1) we get

$$V_{\text{bilayer-Osm}} = \pi [t^2 + 2rt (\alpha)^{1/2}]$$  \hspace{1cm} (3)
Therefore, change in bilayer volume, as a result of change in osmolarity, is calculated by

$$V_{\text{Bilayer-Osm}} - V_{\text{Bilayer}} = 2\pi h r t \left[(\alpha)^{1/2} - 1\right]$$

for hypotonic external solution

and

$$V_{\text{Bilayer}} - V_{\text{Bilayer-Osm}} = 2\pi h r t \left[1 - (\alpha)^{1/2}\right]$$

for hypertonic external solution

(4a)

Now, for E. coli cells, $h \sim 2000$ nm, $r \sim 500$ nm and for the outer membrane $t \sim 3$ nm. If $\alpha = 1.2$ (20% increase in volume in presence of hypotonic solution), then the bilayer volume increases by $\sim 1.8 \times 10^6$ nm$^3$ using Eq. (4a). On the other hand if $\alpha = 0.8$ (20% reduction in volume in presence of a hypertonic solution), then the bilayer volume decreases by $2 \times 10^6$ nm$^3$ using Eq. (4b).

Now, there are four L-rings on an average per bacterial cell. Assuming a cylindrical L-ring (see Fig. 1) with the radius of the cylinder being 10 nm and length of the transmembrane part of the cylinder being 3 nm ($= t$), each L ring has a “resting” volume of $\sim 942$ nm$^3$. Assuming that the change in bilayer volume resulting from external osmolarity is distributed amongst its constituents and the four L-rings are affected equally to accommodate about 0.1% changes in the overall bilayer volume, we find that each of the L-rings becomes “looser” by a volume of $1.8 \times 10^6/4 = 450$ nm$^3$ in case of the 20% increase in cell volume, which translates to loosening of the protein in Figure 6(A) by $[[((942 + 450)/3\pi)^{1/2} - 10] \sim 1.2$ nm (a 20% increase in radius of the cylindrical L-ring from 10 to 12 nm). Similarly for 20% decrease in cell volume, the L-rings become “tighter” by a volume of $2 \times 10^6/4 = 500$ nm$^3$, which translates to tightening of the shell in Figure 6(C) by $[10 - ((942 - 500)/3\pi)^{1/2}] \sim 3$ nm (a 30% decrease in radius of the cylindrical L-ring from 10 to 7 nm). These values are obtained, of course, due to the assumption the L-rings are accommodating only 0.1% of the changes in membrane volumes (with lipid packing becoming looser or tighter while keeping the dimensions of individual lipids the same and other membrane proteins also accommodating some changes) resulting from different osmotic conditions. There must be a threshold value beyond which a protein in the bilayer becomes incompressible. Our data suggests that the flagellar motor becomes incompressible around an external Osmolarity of 1.5 for NaCl (see Fig. 5(A)). This is very similar to the value obtained experimentally at which the volume of the cell becomes insensitive to NaCl osmolarity. Based on the calculations shown and our experimental data, it is clear that while at this osmolarity the flagellar motor does not exactly stall, but it is at its lowest performance level, where, the protein is further incompressible. However, for sucrose, we can clearly see that the motor stalls at an external osmolarity of $\sim 3$ Osm. This means that salt induced osmolarity is not able to exert as much membrane tension as compared to sucrose, possibly due to compensation by ion channels in the case of salt. Rough estimate of the possible compressibility of the L-ring after considering the structure yields that under hypertonic conditions, the motor will stall at a compression of about 30% of the L-ring (i.e., reduction in radius from 10 nm to 7 nm), which makes it equivalent to the radius of the hook, thereby squeezing the hook to render it immobile. This would require the reduction in membrane bilayer volume by $\sim 1.3 \times 10^6$ nm$^3$ which is quite large compared to the 20% example taken above (where reduction in bilayer volume was about 3 orders of magnitude lower). This reduction is as much as $\sim 80$% of the bilayer volume, which would mean that one would expect it at very high osmolarities only, where the cells are expected to shrink considerably. Our data strongly indicate the same (see Fig. 5(B)) as we can clearly see a sharp decline in the % motility around 1.5-2 Osm. Beyond an external osmolarity of 10 times the value of an isotonic solution for bacterial cells (i.e., $\sim 2.4$ Osm), we see stalling of the flagellar motor in terms of negligible % motility.

It is interesting to note that recently, another membrane bound bionanotechnological marvel, the H$^+$-ATPase was found to exhibit a decrease in activity with increasing osmolarity. The authors in this work found a clear inhibition in the activity of the plasma membrane bound ATPase nanomachine with increasing concentrations of trehalose, sucrose, and glycerol. However, the interpretation of the data was to attribute the decrease in the activity of the ATPase to increasing viscosity. The inhibition of ATPase was compared with similar studies done on soluble enzymes, thereby neglecting the role of the plasma membrane specifically for the ATPase. Our work here proposes to interpret the ATPase data also in terms of the observed decrease in activity resulting from “squeezing” of the protein due to tight lipid packing induced by using hypertonic solutions.

Our results clearly indicate that while considering the efficiency of a prototypical biological nanomachine like the flagellar motor, it is pertinent to consider the role of the anchor in which the protein is placed. In fact, it might be inappropriate to consider just the flagellar protein as the bio-nanotechnological marvel, but it is the flagellar protein anchored in a membrane with the right phospholipids density having the right membrane tension that reflects nature’s nano-machinery par excellence. Finally, our results also indicate that the flagellar nano-propellers are not anchored in specific domains of lipids since there is a clear non-monotonous dependence of bacterial motility on overall membrane tension (to which fixed domains would be somewhat insensitive).
Role of Membrane Tension in Flagellar Motion of *E. coli*  

**References and Notes**


Received: 21 June 2006. Revised/Accepted: 27 August 2006.
What is the role of the biological membrane in determining the performance of bio-nanomachines that are anchored in it? We investigate the effects of varying the membrane tension, by providing different extracellular osmotic environments, on the performance of the bacterial flagellar motor. Under hypotonic conditions, we propose that loose packing of the nano-propeller leads to a lower performance which is the effect of tight packing also. We report that the optimal membrane tension for the flagellar motor’s performance is produced by an external osmolarity of 0.5-1 Osmolar, which surprisingly is not the iso-osmolar condition for the bacteria. Our data strongly demonstrate an active role of membrane tension in the nano-propeller driven bacterial motion.