Effect of Surface Chemistry and Young's Modulus on the Surface Motility of the Bacterium *Pseudomonas Aeruginosa*

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Abstract

This study demonstrates that the surface motility of the bacterium *Pseudomonas aeruginosa* is dependent on the surface chemistry of the underlying substrate. In particular, cells on hydrophobic polydimethylsiloxane (PDMS) have a speed that is on average 38% greater than on hydrophilic PDMS. These results were obtained using time-lapse microscopy of bacteria exposed to continuously flowing tryptic soy broth growth medium at 37 ^{o}C. Not only are the mean speeds different, the distributions of speeds are also different: on the hydrophobic substrate, a smaller proportion of bacteria move by less than about one body-length (~3 μm) in 60 minutes. In addition, the surface chemistry affects the orientation of the cells: there is a greater fraction of "walking" bacteria on the hydrophobic surface. Sensitivity to the substrate surface chemistry occurs despite the presence of a complex mix of substances in the growth medium and offers hope that surface chemistry can be used to tune motility and the progression to biofilm formation. Additionally, the effect of reducing the near-surface Young's modulus of the PDMS from 7000 to 70 kPA is investigated. For the lower modulus material, there is an increase in the likelihood of a bacterium executing sudden, high angle turns. This is evident in images with a framerate of one frame per 0.22s. However, the impact of these turns is averaged out over longer times such that the mean speed over periods of more than about one minute is the same for bacteria on both the high and the low modulus materials. Consequently, except over very short time intervals, Young's modulus in the surface region is not effective as a means of modulating motile behavior.

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General Audience Abstract

This study demonstrates that the ability of the bacterium *Pseudomonas aeruginosa* to move on a solid surface is dependent on the surface chemistry of the underlying substrate. In particular, cells on hydrophobic polydimethylsiloxane (PDMS) have a speed that is on average 38% greater than on hydrophilic PDMS. These results were obtained using time-lapse microscopy of bacteria exposed to continuously flowing growth medium at 37 ^{o}C. Not only are the mean speeds different, the distributions of speeds are also different: on the hydrophobic substrate, a smaller proportion of bacteria move by less than about one body-length (~3 μm) in 60 minutes. In addition, the surface chemistry affects the orientation of the cells: there is a greater fraction of vertically-oriented bacteria on the hydrophobic surface. Additionally, the effect of reducing the stiffness of the PDMS from 7000 to 70 kPA is investigated. For the less stiff material, there is an increase in the likelihood of a bacterium executing sudden, high angle turns. This is evident in images with a framerate of one frame per 0.22s. However, the impact of these turns is averaged out over longer times such that the mean speed over periods of more than about one minute is the same for bacteria on both the high and the low stiffness materials. Consequently, except over very short time intervals, stiffness in the surface region is not effective as a means of changing patterns of surface-bound *P. aeruginosa* movement.

Acknowledgements

To Chris Pritchett and Studio Collective –

You showed me that I cannot live without access to creative expression. If not for people like you, academia would have long since ossified into its own sarcophagus. Never allow yourself to become boring.

To Brant Thomas, Logan Hodges, Jimmy Ivory, and Kevin Bowen –

Mike Tyson said, “Everybody has a plan until they get punched in the mouth.” I’m sure that’s true for most people, but not for me – I didn’t have a plan until I got punched in the mouth. In the year I spent practically living in your gym, I came to understand boxing as something much more than simply a display of brutality. Boxing is complex, with a strategic depth that rivals chess. It is also shockingly hard on the body, so much so that it’s difficult not to have some measure of respect for anyone who steps into the ring more than once.

I’ve spent more than a thousand hours training. I’ve sparred with professionals. These are probably my greatest accomplishments. Perhaps most importantly, I now have a plan – to train hard, because the other guy is, too.

To Lynne –

For reasons I can’t express with words.

To my advisor –

For your grace and patience.

Table of Contents

List of Figures

List of Tables

List of Equations

Chapter 1. Introduction

1.1 Objective and application

The objective of this work is to determine whether, and to what extent, the motility of surface-bound *Pseudomonas aeruginosa* can be influenced by the hydrophilicity and surface stiffness of the underlying substrate. In particular, two hypotheses are investigated:

1. *Pseudomonas aeruginosa* motility increases as substrate Young's modulus increases.
2. *Pseudomonas aeruginosa* motility can be modulated as a function of surface chemistry.

The substrate chosen for this investigation is PDMS (polydimethylsiloxane), a biocompatible organic polymer. PDMS allows for conveniently tuning the stiffness of the substrate by controlling the extent of crosslinking and the presence of additives such as silicone nanoparticles. The hydrophilicity of the PDMS substrate is also easy to change – although PDMS ordinarily is highly hydrophobic, it can be made hydrophilic by processing through an oxygen plasma cleaner. This work is intended to inform the design of surface coatings for materials in clinical settings, with the ultimate goal of impeding biofilm formation.

The motility of *P. aeruginosa*, and some other bacteria, is achieved by appendages such as type IV pili that must adhere to a solid. These adhesive interactions may be influenced by the surface chemistry of the substrate. This is the basis of the hypothesis that the surface chemistry of the solid may affect the motility. There is also a possibility that interactions of the body of the bacterium with the solid affect motility.

With regard to surface stiffness, it is hypothesized that *P. aeruginosa* will move more rapidly on a stiffer surface. As discussed in following sections, bacteria may be capable of deforming a non-rigid surface by means of one or more appendages anchored in the cell membrane. Less stiff surfaces will deform more easily, and the metabolic energy provided by the cell and used to deform the surface effectively will be wasted. Therefore, the bacteria may undergo slower or less efficient motion on less stiff solids.

1.2 Biofilms

Biofilms are three-dimensional structures of microbial cells encased within a matrix of extracellular polymeric substances (EPS). They commonly grow at solid–liquid interfaces.[1] Typically, biofilms develop through a series of discrete phases. Following initial adhesion of bacteria to a surface, biofilm-forming bacteria will reproduce and explore the surface using certain modes of motility.[2] As this process continues, the bacteria undergo quorum sensing, wherein they are able to detect the presence of various substances secreted by other bacteria inhabiting the surface.[3] This allows the bacteria to modulate their motile behavior in order to agglomerate together into increasingly large groups. Once this agglomeration reaches a critical threshold, a phenotypic change occurs within the bacteria.[4] This results in the secretion of extracellular polymeric substances (EPS) which together with the bacteria form a three-dimensional structure known as a biofilm.

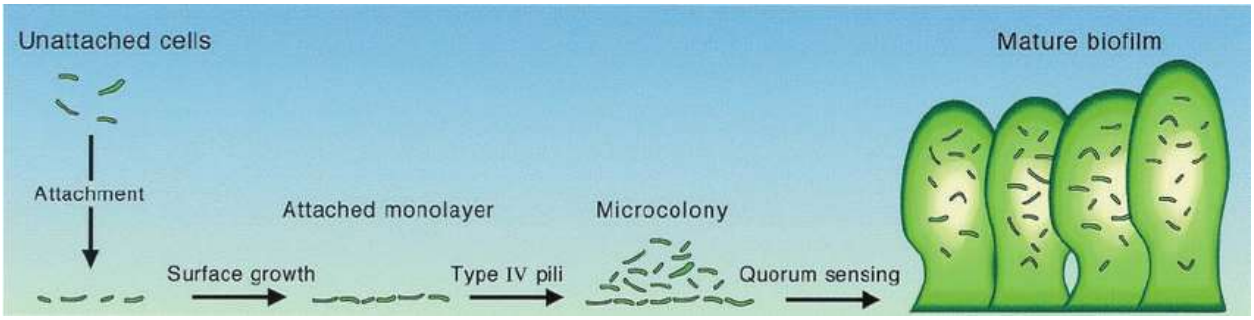


Fig. 1. Biofilm formation proceeds according to several phases, beginning with adhesion of cells to a substrate and ending with the production of a three-dimensional structure. Reproduced with permission from Parsek, M. and E. Greenberg (2000). "Acyl-homoserine lactone quorum sensing in Gram-negative bacteria: A signaling mechanism involved in associations with higher organisms." Proceedings of the National Academy of Sciences of the United States of America **97**: 8789-8793. Copyright (2000) National Academy of Sciences, U.S.A.

Biofilms present a variety of challenges to humans, including medical infections and the fouling of pipes and ship hulls. In 2015, there were about 700,000 hospital-acquired infections in the United States; such infections are often associated with biofilms that are attached to indwelling medical devices such as catheters.[5-6] It is also notable that biofilms are often resistant to antibiotics. Extracellular polymeric substances (discussed in the following section) protect the bacteria and can complicate treatment options for patients.

Each stage of biofilm development presents opportunities to disrupt the process. Most simply, a biofilm cannot form if bacteria cannot adhere to a surface. Considerable research effort has been directed toward surface-chemical approaches to prevent bacteria from adhering to solids. Much of this effort is focused on chemical coatings, such as polyethylene oxide, intended to inhibit adsorption.[7-9] Although highly effective, this approach can be defeated by adsorption of relatively thin layers of material. Such material may be present in a liquid that contacts the surface, or may be secreted by a bacterium. Other chemical approaches to the problem include impregnating the implant with antimicrobials that leach out over time.[10] Recently, the Aizenberg group has shown that certain liquid-infused surfaces can resist biofouling.[11-13] In other recent work, the Ducker group has shown that spherical surface features can reduce the number of adsorbed cells by 99%.[14]

A second method is to prevent bacteria from growing or to cause them to disperse on a surface, so as not to reach the critical concentration at which biofilm development begins. Several groups have examined the effect of topography on surface growth or arrangement.[15-20] Recent work in the Ducker group has shown that the surface motility of the bacterium *P. aeruginosa* can be reduced using surface topography.[21] The general conclusion is that micrometer-scale features can be used to inhibit surface growth, and that topography affects the position of adsorption.[19-20, 22]

In the current work, the effect of surface chemistry and modulus on surface motility is examined. Clearly, the adsorption of bacteria is important, and has justifiably been well studied. But, on the path to biofilm formation, many species actively move across the surface to find each other, form colonies and later biofilms.[23] Such active movement is termed "motility." Means of inhibiting motility of surface-bound, biofilm-forming bacteria have not been as extensively explored.

One example of a surface motile bacterium is *Pseudomonas aeruginosa*, an opportunistic human pathogen. *P. aeruginosa* is a rod-shaped, gram-negative bacterium, approximately 3 μm in length. In particular, it is well known for infecting patients suffering from cystic fibrosis.[24]

In addition to preventing adhesion, there are opportunities to interrupt biofilm formation by inhibiting surface motility, colony formation and transition to a biofilm. There is a known link between biofilm formation and the presence of appendages associated with surface motility in *P. aeruginosa*,[25] and this is an area of active research. *P. aeruginosa* achieves surface motility primarily by means of an appendage known as a type IV pilus (TFP).[26]

1.3 Type IV pili

Some bacteria are capable of inhabiting either the body of a fluid or an interface with a solid object. In the fluid (planktonic) state, bacteria may utilize a variety of means to translocate, including the well-known helical flagellum. Through mechanisms that are the subject of current research, bacteria may move between the planktonic and surface-bound states.[27] Of particular interest in this work are the modes of motility utilized in the surface-bound state. *P. aeruginosa* may employ a number of different appendages, possibly including the flagellum, in the process of moving relative to a surface with which the bacterium is in contact. It is widely believed that the appendage most directly related to surface-bound motility is the type IV pilus.[28-29]

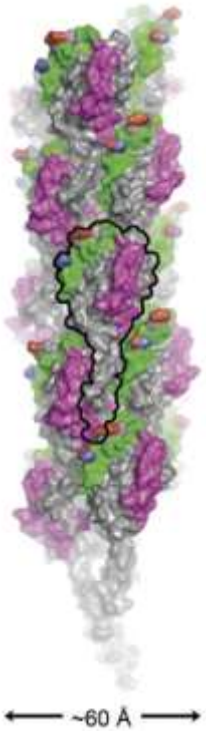


Fig. 2. Schematic representation of a type IV pilus, with a representative pilin subunit outlined in black. Pilin is added to the filament in a helical pattern. Reproduced with permission from Craig, L. L., Juliana (2008). "Type IV pili: paradoxes in form and function." Current Opinion in Structural Biology.

Type IV pili are long (multiple microns), thin (6-9 nanometers radius) fibrous structures which the bacterium projects outward from the bacterial membrane. The bacterium synthesizes the protein, pilin (also known as pilA), which can be considered as the monomer from which the pilus is constructed. The pilus is assembled as it is projected outward. After the tip contacts a solid, the length of the pilus is reduced by removing pilin units near the cell membrane. This disassembly is accompanied by an increase in tension on the pilus which moves the bacterium toward the contact point of the pilus with the solid.[30-31] To use an analogy, imagine a person floating in a pool of water. If a rope, anchored at some point outside of the pool, is thrown to this person, they can use their arms to exert tension along the rope and pull themselves in the direction of the anchoring point. This is quite similar to the way bacteria use type IV pili to drag

themselves along a surface. Note that this is only one of several known functions served by the type IV pilus. They are also involved in the formation of microcolonies.

These operations are supported by a complex of proteins at the base of the pilus, including the protein pilT, which acts as a motor to retract the filament. A hexamer composed of six distinct subunits, pilT is capable of retracting and depolymerizing the filament. By doing this, the bacterium exerts tension on the pilus. Because part of the type IV pilus is adhered to a solid surface, the exertion of tension pulls the bacterium forward.

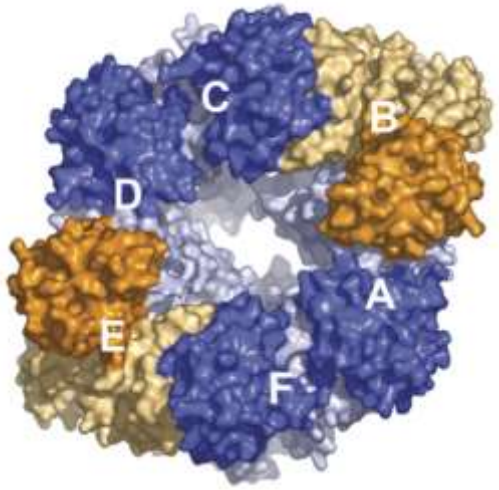


Fig. 3. pilT protein, with each of the six units of the hexamer denoted with a letter. pilT is among the most powerful biological motors known to exist; it can exert forces of approximately 100 pN. Reproduced with permission from Craig, L. L., Juliana (2008). "Type IV pili: paradoxes in form and function." Current Opinion in Structural Biology.

At the other end of the pilus lies the tip pilin. Distinct from pilA, the tip pilin unit (pilY1 in *P. aeruginosa*) may be particularly relevant for understanding motility. The pilus adheres to a surface through a mechanism that is not well understood. It seems logical to the author that the pilus adheres either at the tip or by lying flat against the surface for some length, or perhaps by some combination.

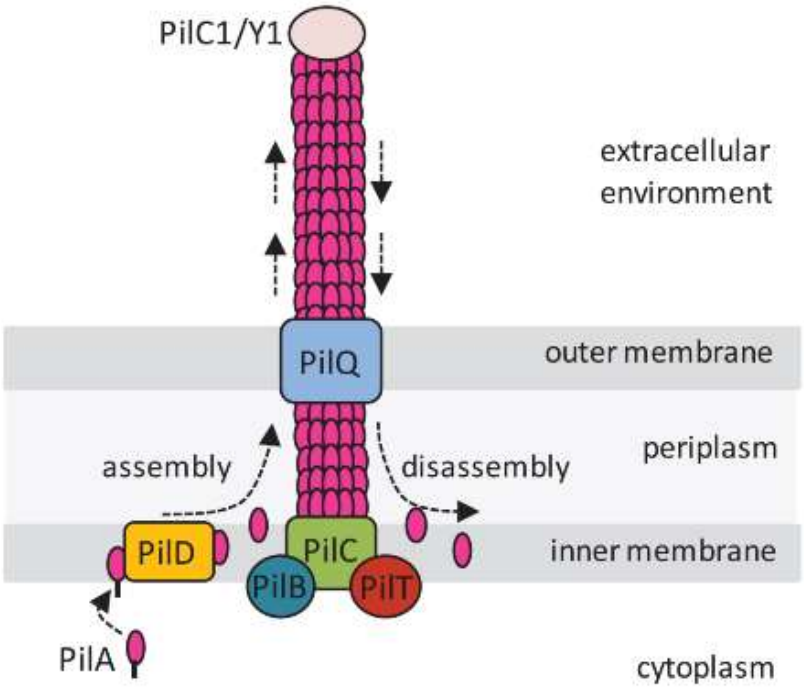


Fig. 4. Complex of proteins that both anchor the type IV pilus to the bacterial membrane and also assemble and disassemble the pilus. This provides for the essential functions of the pilus: to extend outward and adhere to a solid surface, and then to bear tension during the disassembly process. Note that this is a simplified model. Reproduced in accordance with MDPI open-access permissions policy from Burdman, S., et al. (2011). "Involvement of Type IV Pili in Pathogenicity of Plant Pathogenic Bacteria." Genes.

1.4 Adhesion

Adhesive interactions between the bacterium and the substrate are mediated by appendages, such as type IV pili, which adhere to the surface, and also by the bacterial membrane. These, in combination with substances known as adhesins, which may be secreted by the cell, result in a complex multitude of adhesions. Due to the complexity of the biomolecular composition of these materials, it is difficult precisely to characterize the physical chemistry governing interactions with the substrate.

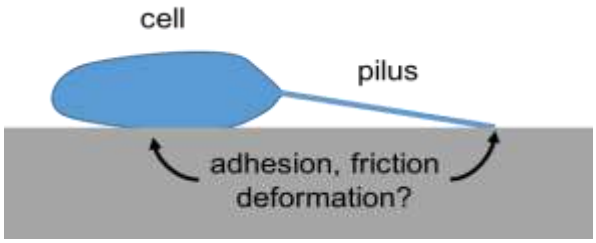


Fig. 5. Sites where surface chemistry or modulus may affect motility.

1.5 Modes of motility

Earlier publications reported that *P. aeruginosa* has two modes of motion: crawling, and walking (see Fig. 5).[32] Between these, crawling has the longest persistence length and thus produces greater net displacements.

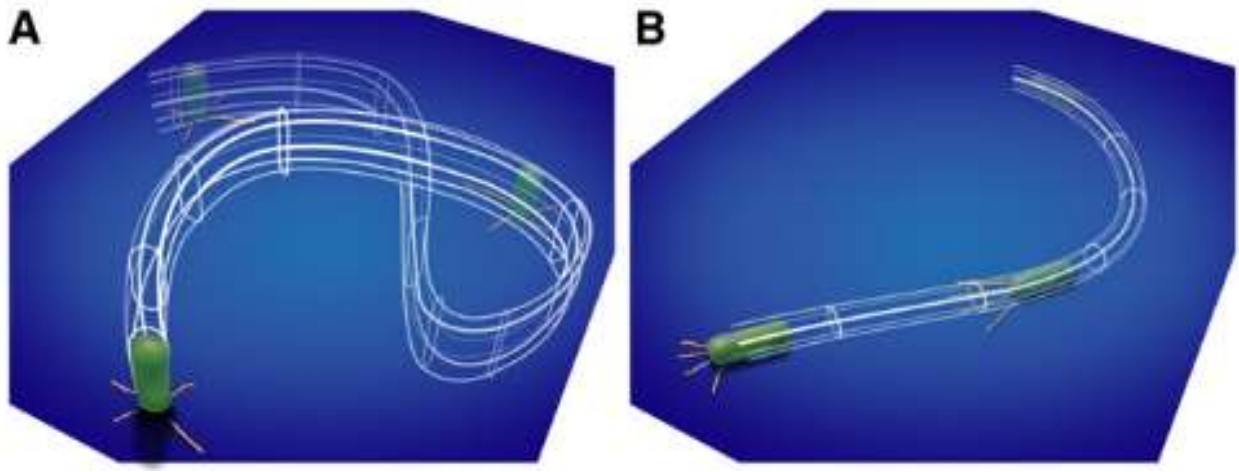


Fig. 6. Overhead view of bacteria (A) walking and (B) crawling. In these experiments, walking bacteria appear as circles or ellipses of very low aspect ratio, whereas crawling bacteria appear as rods, which can be approximated as ellipses of higher aspect ratio. Reproduced with permission from Conrad, Jacinta C., et al. (2011). "Flagella and Pili-Mediated Near-Surface Single-Cell Motility Mechanisms in P. aeruginosa." Biophysical Journal **100**(7): 1608-1616.

1.6 Effect of surface chemistry

Recent studies have shown that surface motility is sometimes directed by extracellular factors; cells may tend to follow trails consisting of polymers secreted from other cells.[33] It has also been demonstrated that there are a variety of modes of motion including crawling (long axis perpendicular to the surface normal), walking (long axis parallel to the surface normal),[32] and slingshotting (rapid changes in the direction of long axis perpendicular to the surface normal).[34]

Although there is an extensive body of work on the effects of chemistry on surface adhesion, there is a notable absence of work on the effect of surface chemistry on motility. We believe there is good reason for surface chemistry to affect motility. First, there is the empirical evidence provided by Zhao et al.

showing that excretion of a slime trail by bacteria affects the direction of surface motion.[33] This suggests that human efforts to alter the solid surface, with a coating or through adsorption, could also affect motility. Second, it is conceivable that surface chemistry could influence the necessary adhesion of the pilus to a solid and the friction between the cell membrane and a substrate (see Fig. 6). There is already ample evidence that surface chemistry affects bacterial adhesion and that adhesion and friction are related.[35-36] However, it is important to note that the initial adhesion events and the processes of friction and adhesion during motion are temporally separated for each adsorbing bacterium. Consequently, the biochemical state of the cell may be quite distinct between initial adhesion and subsequent motility.

1.7 Effect of surface modulus

Surface modulus has been shown to affect the modes of motility of *P. aeruginosa*.[37-38] A recent study found that *Escherichia coli* is more motile on stiffer PDMS surfaces.[37] Using motility mutants, the authors concluded that in this case the motility was mediated by flagella. It is not immediately clear why surface modulus should affect motility mediated by type IV pili. One possibility is that, should the solid be sufficiently low in stiffness, then the rate of solid deformation might approach the speed of the pilus, thereby slowing the displacement of the cell body relative to the laboratory frame of reference.

Another study found that *P. aeruginosa* undergoes slingshotting more frequently on less stiff surfaces at 30 ^{o}C, but not at 37 ^{o}C.[38] Although the authors also investigated the effect of temperature, this study serves as further motivation to investigate the effect of surface modulus on *P. aeruginosa* motility.

1.8 Chapter 1 summary

In the interest of studying means to mitigate the formation of biofilms on surfaces, one possible avenue involves limiting the motility of surface-adhered bacteria. Some bacteria, such as *P. aeruginosa*, utilize appendages known as type IV pili to move across a surface. The interactions between the solid substrate and the bacterial membrane as well as the type IV pili present opportunities to disrupt the ordinary motile

behavior of such bacteria. Particularly, there is reason to believe that it might be possible to manipulate both the Young's modulus in the surface region and the chemistry of the surface to cause a change in motile behavior.

Chapter 2. Experimental

To investigate how surface Young's modulus and chemistry may influence the motility of biofilm-forming bacteria, it was necessary to specify the organism (and associated growth conditions), the substrate, and the imaging equipment.

2.1 Preparation of solid samples (substrata)

In this work polydimethylsiloxane elastomer (PDMS) was used as the substrate for all of the experiments to investigate the effects of surface chemistry and modulus. PDMS is widely used in both laboratory settings, for biological applications, and in catheters ,[39-41] and has been shown to affect the release of cells.[42] PDMS also allows for varying both the surface wettability and the Young's modulus with a single material type.

The surface chemistry of PDMS was varied by exposure to oxygen plasma: the native surface is hydrophobic with a water contact angle of about 100° whereas the plasma-treated surface is hydrophilic with a water contact angle close to zero. In this work, the Young's modulus was altered using a method developed by Palchesko,[43] which involved mixing together different proportions of two formulations of PDMS, Sylgard® 184 and Sylgard® 527, that have very different moduli due to different concentrations of cross-linking groups. This method is preferable over the more common method of changing the ratio of ingredients in a single formulation, because changing the ratio of ingredients may leave unreacted monomer.[43] It is possible that unreacted monomer will behave similarly to low molecular mass silicone, which has been shown to affect bacterial adhesion.[11-13] Using the mixture of two formulations, substrates spanning an order of magnitude in the bulk Young's moduli (25–250 kilopascals) were produced. This range spans the lower end for materials that can hold their shape when handled, and is similar to moduli characteristic of some physiological tissues.[44]

All solids were fabricated from the Sylgard products (PDMS), Sylgard® 184 and Sylgard® 527. Sylgard® 184 consists of dimethylsiloxane; tetra(trimethylsiloxy)silane; dimethylvinylated and trimethylated silica; dimethyl, methylhydrogen siloxane; and tetramethyl tetravinyl cyclotetrasiloxane.[45] Sylgard® 527 contains neither the silica nor the branched or cyclic components; these components are designed to cause cross-linking and a greater modulus.

2.1.1 Variation of wettability

Sylgard® 184 was prepared by mixing, in a 10:1 ratio, the base and the curing agent and then spin-coating onto glass cover slips. The polymer-coated cover slips were then heat-treated in an oven at 150 ^{o}C for 48 to 72 hours, following the procedure of Eddington et al.[46] The resulting PDMS films were approximately 7–10 μm in thickness. The heat-treatment is intended to (*i*) cure the polymer and (*ii*) evaporate components that are more volatile. The advancing water contact angle was 100° after heat treatment. At this point, half of the samples were treated with an oxygen plasma at 100 W for approximately 5 minutes at a pressure of less than 200 mTorr; this was intended to produce surface hydroxyl groups and thereby render the surface hydrophilic. After plasma treatment, the surfaces were wetting, meaning the advancing water contact angle was <10°. The contact angle remained at <10° for at least one day when the solid was left in air or water. Without the heat treatment, the water contact angle would revert to about 100° within one day; this process is known as recovery.[47] It is believed that this is due to migration of low molecular weight components, which are hydrophobic, from bulk to the surface. Layers of oil have been shown to affect bacterial motility;[33] as such, it was necessary to eliminate the possibility that the amount of oil on the surface of our solids could be a confounding factor.

2.1.2 Variation of modulus

The components of Sylgard® 184 and Sylgard® 527 were separately mixed and the two formulations were mixed together. Sylgard® 527 has a lower modulus than Sylgard® 184, so by controlling the mass ratio of

527:184, it was possible to alter the Young's modulus. Table 1 shows results from two different measures of modulus:

1. Elastic modulus of the surface measured via the indentation of an atomic force microscope probe (0.1 μm radius, Nanotools) at 30 °C.[48] At least 25 measurements were taken for each substrate.
2. Shear modulus of a bulk sample (thickness 1 mm) measured using a TA Instruments AR-2000 rheometer at a strain of 0.01 at 35 °C and a frequency of 1 Hz. Shear moduli were then converted to Young's moduli by treating the PDMS as a perfect elastic solid. At least ten measurements were taken for each substrate.

AFM force–indentation data were used to calculate modulus via Sneddon's model; sample data and fit are shown in Fig. 7.[48] In the following equation, Force, F (nN), is related to indentation depth, δ (nm). The parameter α relates to the geometry of the cantilever tip, and ν is the unitless Poisson's ratio of the material (very close to 0.5 for PDMS).[49]

$$F = \frac{2}{\pi}\tan(\alpha)\left(\frac{E}{1-\nu^2}\right)\delta^2 \qquad (1)$$

As the AFM cantilever tip indents into the surface, both the force and the indentation depth are measured. This allows for calculating the Young's modulus, E. The resulting curve of force as a function of indentation is parabolic in shape.

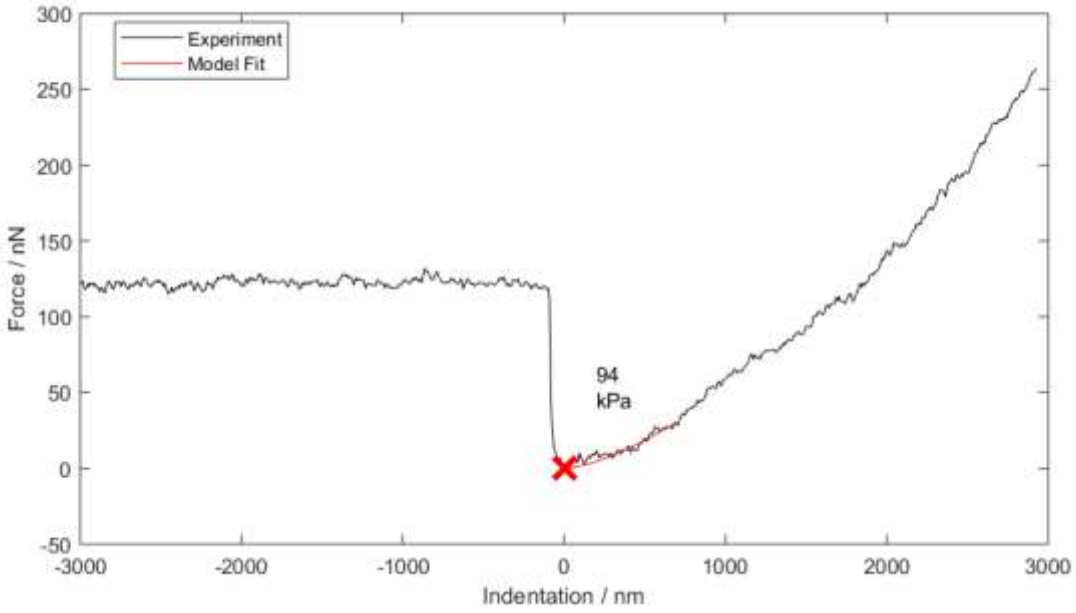


Fig. 7. Example of curve fitting to AFM force-indentation data. Note that the model is most valid at the point of indentation. As indentation increases, the model loses accuracy. Similar curves were produced for substrates of each formulation.

The results confirm a large decrease in both bulk and surface modulus through addition of the 527 formulation. The two measurements were not in quantitative agreement, which is not surprising since they measure different quantities in different parts of the sample. That is, using the atomic force microscope, the surface modulus was measured. Due to the bonding in the surface region (which is exposed to liquid water), it is reasonable to expect that the surface modulus could be significantly different from the modulus in the bulk of the material. A recent paper by Kolewe et al.[50] also reports that the measured bulk value differed significantly from the AFM indentation measurements for a stiffer polymer (a hydrogel). They also report that the stiffness measured by AFM indentation was not a strong function of the film thickness. The AFM measurements utilized samples that were prepared in the same

way as the samples exposed to bacteria. These measurements probe the surface rather than the bulk properties, and are likely to be more indicative of the range of moduli experienced by the bacteria.

Table 1. Measured Young's modulus of PDMS mixtures.

527:184 ratio	**Modulus / kPa**	
	Surface	**Bulk**
0:1	6.7×10^3	250
5:1	105	43
8:1	69	25

2.2 Bacterial growth

A mutant of *P. aeruginosa* PA01 was provided by Prof. Joe Harrison of the University of Calgary.[33, 51] The mutant had constitutive expression of the tdTomato fluorescent protein, to facilitate imaging, and resistance to the antibiotic gentamicin. Bacteria were streaked onto tryptic soy agar plates and incubated at 37 ^{o}C overnight. This is a common incubation method for a variety of bacteria.[12, 52] One colony from the resulting incubation was used to inoculate 50 mL of 30 g/L tryptic soy broth (100% TSB)[53] in a 250 mL baffled flask. The flask was incubated (211DS, Labnet) at 37 ^{o}C and shaken at 300 rpm for approximately 24 hours. Thirty µg/mL gentamicin (Fisher Scientific) was added to all growth media. Following incubation, a new culture was established in 50 mL of tryptic soy broth with 50 µL of inoculum from the earlier liquid-medium culture and was left to incubate for four hours such that the bacterial growth was in exponential phase. An aliquot was then removed and diluted to OD_{600} = 0.01 in TSB. This aliquot was used to inoculate the flow cell. BD TSB was obtained from Fisher Scientific, USA, and contains the following ingredients: peptone, NaCl, K_2HPO_4, dextrose and glucose. The peptone contains a peptide/amino acid-rich digest of casein and a carbohydrate-rich digest of soy.

2.3 Live-cell imaging

Cells were imaged in brightfield and fluorescence with an upright Zeiss Imager.M2 microscope using a 63x oil immersion objective with a numerical aperture of 1.4. Images with a framerate of one frame per 30 seconds were taken with a Zeiss Axiocam 506 mono camera with 5x5 binning (effectively 544 x 440 pixels). Using these settings, the bacteria were approximately 10 pixels in length. Prior work showed that phototoxicity did not affect motility under these conditions, and that the tDTomato strain behaved similarly to the wildtype.[21] tDTomato has an excitation wavelength maximum of 554 nm and an emission wavelength maximum of 581 nm. Additional high framerate experiments were performed at one frame per 0.22 seconds for 30 minutes.

2.4 Flow-cell experiments

The imaging equipment occupied a plexiglass enclosure, the interior of which was held at a temperature of 37$\pm$1 ^{o}C. Flow cell experiments were identical to those described in reference [21], which were based on work in references.[32, 54]100% TSB medium was pumped through each flow cell channel at 4 mL/hour for several minutes until steady-state flow conditions were established. The purposes of this flow were to provide nutrients for the bacteria and to remove waste material from the flow cell. Flow was interrupted and 250 µL of inoculum was injected into the fluid streams immediately upstream of each flow cell. The flow cells were then flipped upside down to allow bacteria, under the influence of gravity, to adhere to the exposed substrate surface. The flow cells were returned to an upright state after 15 minutes before ordinary flow conditions were reestablished with a flow rate of TSB of 4 mL/hour, and then the time-lapse video was recorded for 60 min. The effect of the fluid flow was examined by measuring the mean bacterial displacement over 5 min. The average absolute value of the displacement of the bacteria in 5 minutes was about 4 µm. In comparison, the average displacement in the *x*- and *y*-directions were –0.31 µm and –0.56 µm respectively on the 184 substrate and –0.37 µm and 0.18 µm on the 8:1 mixture, where the *x* direction is the direction of flow. This indicates little directional influence from flow or other anisotropic effects. A

typical division time for a bacterium on the solid was 30 minutes. This was determined by measuring the cell length as a function of time and attributing the period between steep declines in length to the time to divide.

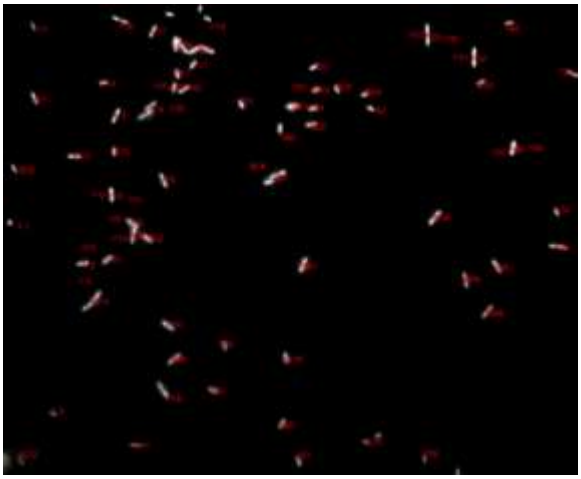
2.5 Particle identification, tracking and speed computation

A combination of thresholding and band pass filtering was employed to alter images to facilitate identification of the bacteria adhered to the surface. To identify and track the bacteria, particle identification and tracking software consisting of Matlab scripts based on an algorithm by Grier and Crocker were used.[55] The centroid (center of intensity) was identified for each bacterium and then the trajectory of each bacterium was tracked by minimizing the sum of the local centroid displacements. The output consisted of a matrix of position data as a function of time for each identified bacterium adhered to the surface. Data for bacteria that were not tracked for at least five minutes were discarded.

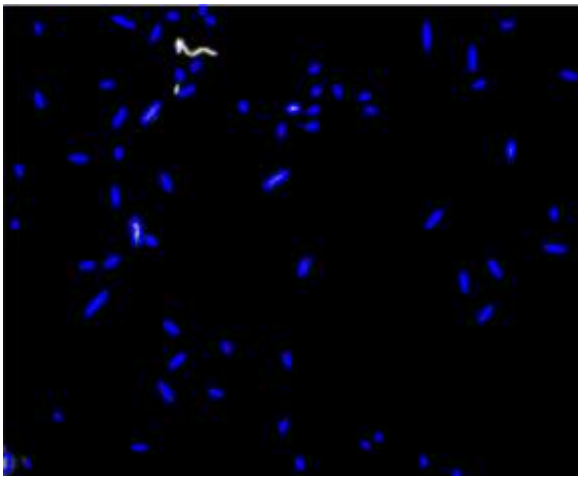
For each remaining bacterium, an average speed for each five-minute (eleven frame) interval in the experiment was calculated, e.g. from frame 1 to 12, 2 to 13, and 3 to 14. This produced a set of several thousand measured speeds for each substrate sample. The statistical significance of differences in the averages was assessed using Student's T-test.

The data for the following figures utilized fits of an ellipse to the image of each bacterium in each frame. Ellipses were determined using MATLAB code that calculated the ellipse with the equivalent second moment to the binary image. From the ellipse, the angle of orientation, the aspect ratio and the centroid of each bacterium were determined.

1. Fluorescence images were taken at regular intervals.



2. A number of scripts were used to perform identification of the bacteria. Identification numbers are shown.



3. Ellipses were fit to each identified bacterium and particle tracking software was used to track each bacterium for the duration of the experiment.

Fig. 8. Overview of the identification, fitting, and tracking procedure. Fluorescence images were used in order to take images with sufficient contrast for subsequent processing.

Chapter 3. Experimental Results

3.1 Bacterial motility is a function of substrate wettability

A pulse of *P. aeruginosa* was introduced into each of a pair of flow channels containing either the hydrophobic or the hydrophilic solids, and the bacteria were allowed to adsorb. While there was a flow of nutrients, time-lapse images were recorded at a rate of one frame per 30 s over a period of 60 min. The video was analyzed to determine how far each bacterium moved in each five-minute period and thus to obtain the speed over 5 minutes. The mean value of this speed was then calculated for each of three experiments (biological replicates), which are shown in Table 2. The mean speed was 38% greater (with a 95% confidence interval of ±24%) on the hydrophobic solid, and a paired, two tailed Student's t-test yielded p = 0.05. This effect can be seen qualitatively in Fig. 9. For each pixel in Fig. 9, the maximum intensity has been plotted over the course of the experiment, thereby indicating all the pixels in which part of a bacterium dwelt at some point in the video. The main qualitative difference in behavior is that the bacteria visited a greater fraction of the hydrophobic solid.

Table 2. Mean speeds over all 5 min intervals for *P. aeruginosa* on hydrophilic and hydrophobic PDMS substrates, with 95% confidence intervals over the means.

	Mean speed / μm/min	
Replicate	**Hydrophilic**	**Hydrophobic**
1	0.59	0.97
2	0.81	1.03
3	0.63	0.81
Average	0.68	0.94
95% CI	±0.13	±0.13

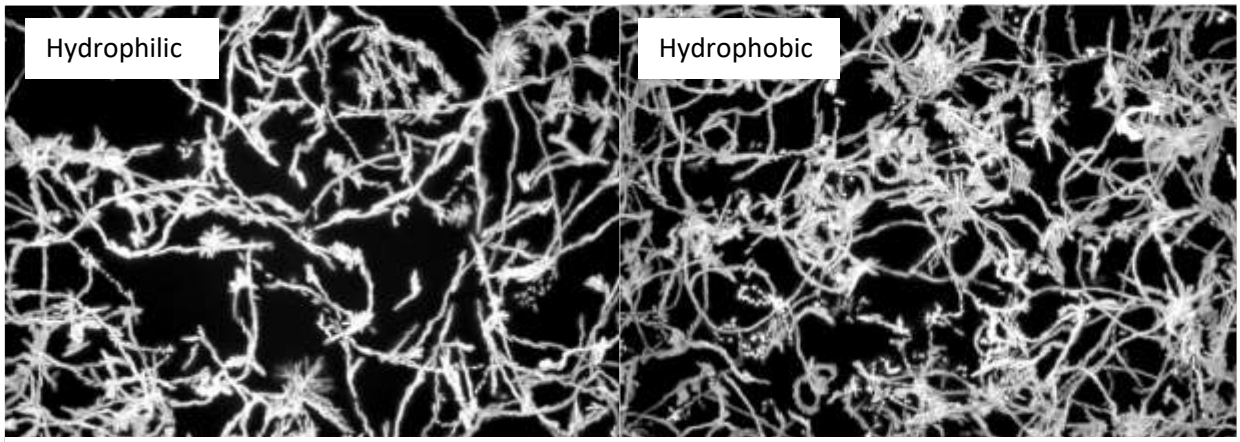


Fig. 9. Comparison of *P. aeruginosa* motility on hydrophobic and hydrophilic PDMS. The images show the maximum intensity of fluorescence in each pixel over a period of 60 minutes and thus indicate all the positions that the bacteria have visited. Qualitatively, there is more intensity in the hydrophobic image than in the hydrophilic image. Setting a threshold of 40% of the maximum intensity, 32% of the image of the hydrophilic solid exceeds the threshold whereas 42% of the image of the hydrophobic solid exceeds the threshold.

It is also interesting to consider the frequency distribution of bacterial speeds on each solid. As shown in Fig. 10, a greater fraction of bacteria has zero or very slow speed (0–0.3 μm/min) on the hydrophilic solid (p = 0.002). These bacteria undergo a diverse range of behaviors, including spinning around one pole, tilting, twitching in place, and division. These three activities are registered as small displacements and are included in the histogram, yet, over longer times such motions do not lead to substantial motion, i.e. displacement by much greater than the length of the bacterium. Note that it is possible for a single bacterium to contribute to multiple regions of the curve. If for example, a bacterium is very slow during one particular interval, and subsequently moves very rapidly, then that bacterium will contribute to probabilities of both low and high speeds.

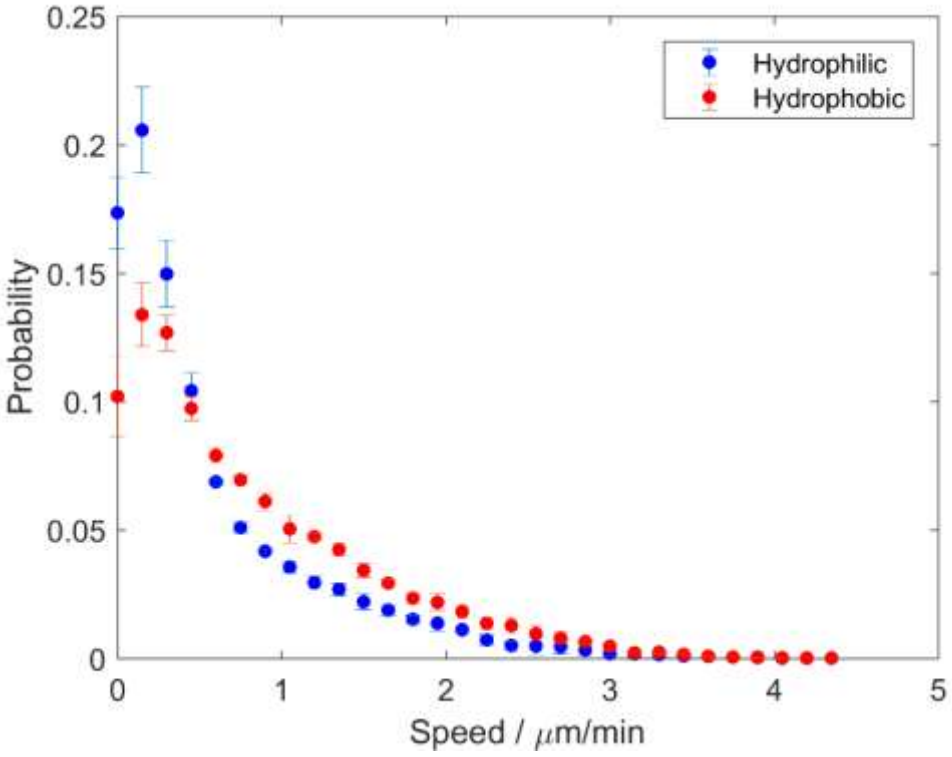


Fig. 10. Probability / (min/μm) of *P. aeruginosa* speeds over a period of 5 min, compared for hydrophilic and hydrophobic solid substrates. Data collected over 60 min; error bars represent ± one standard error.

After inspecting the images, it was noticed that a primary difference between motility on the two solids had to do with different proportions of bacteria that did not undergo substantial displacement over the course of the experiment. To quantify this difference in motility on the two solids, the following metric was chosen: the fraction of bacteria that moved by less than 3 μm (a distance that is about one body length) *over the 60 minutes of the experiment*. The results are shown in Table 3. About twice the fraction of bacteria (9% vs 4%) move <3 μm on the hydrophilic sample (Student's t-test, single tailed, p = 0.03). Thus, a greater fraction of the bacteria on the hydrophilic surface remains in approximately the same position over the course of the experiment, and this is likely the reason for the lower mean speed.

Table 3. Proportions of bacteria that have a total displacement < 3 μm over 60 min.

	Proportion of bacteria with total displacement < 3 μm	
Replicate	**Hydrophilic**	**Hydrophobic**
1	0.08	0.04
2	0.07	0.02
3	0.13	0.06
Average	0.09	0.04

3.2 Young's modulus does not have a large impact on average motility over several minutes

The role of the Young's modulus of a solid in influencing bacterial motility on a hydrophobic solid was also considered. Because *P. aeruginosa* uses retraction of type IV pili to move along a surface, any deformation of the solid toward the bacterial body under the load of the pilus reduces the displacement of the bacteria in the laboratory frame. In addition, any energy the cell expends by deforming the surface is energy that does not directly contribution to cell motion. On this basis, it is possible that bacteria could move more slowly on lower-modulus surfaces. The flow cell used in this experiment enabled study of two moduli simultaneously, resulting in two paired comparisons: high–low and high–medium moduli, the results of which are shown in Tables 4 and 5. The effect of modulus on mean speed is numerically small and is not significant (p = 0.44). Probability distributions of speeds are available in Appendix A.

To determine whether the linear speeds of the bacteria were time-dependent in these experiments, separate distributions of speeds were plotted for all data collected during the first and second halves (30 min each) of the experiments. The distributions did not appear to be significantly different, indicating that the linear speeds of the bacteria are time-invariant in these experiments.

Tables 4 and 5. Mean speeds of *P. aeruginosa* on solids of different moduli. A two-factor ANOVA over all replicates gives p = 0.44. Individual ANOVA tests between either pair of substrates also indicate no statistical significance.

Mean speed / µm/min

Replicate	Higher modulus (6,700 kPa)	Lower modulus (69 kPa)
1	0.58	0.57
2	0.66	0.74
3	0.77	0.90
Average	0.67	0.74
95% CI	±0.11	±0.17

Mean speed / µm/min

Replicate	Higher modulus (6,700 kPa)	Lower modulus (105 kPa)
1	1.03	0.89
2	0.53	0.70
3	0.61	0.84
Average	0.72	0.81
95% CI	±0.30	±0.11

3.3 Effect of Young's modulus on short-duration events

Previous work has described a phenomenon called a slingshot, which is characterized by large combined angular and translational speeds of a surface-bound bacterium over a short duration.[34, 56] Such events alternate with longer-duration periods in which the angular and translational speeds are low.[34] Slingshots are believed to occur when a bacterial pilus lets go of the solid surface, such that there is suddenly a large net tension from pili remaining on the surface. High angular speed events occurred in < 0.1 s and repeated every 0.2 – 6 s.[56] Zhang et al. observed both a larger mean squared displacement (MSD) and a lower rate of slingshotting on a collapsed brush than on an extended brush (which had a lower modulus). The authors attributed the higher MSD to the lower rate of slingshotting because the slingshots caused a change in direction. These changes in direction impeded linear motion, which contributes to the mean squared displacement.

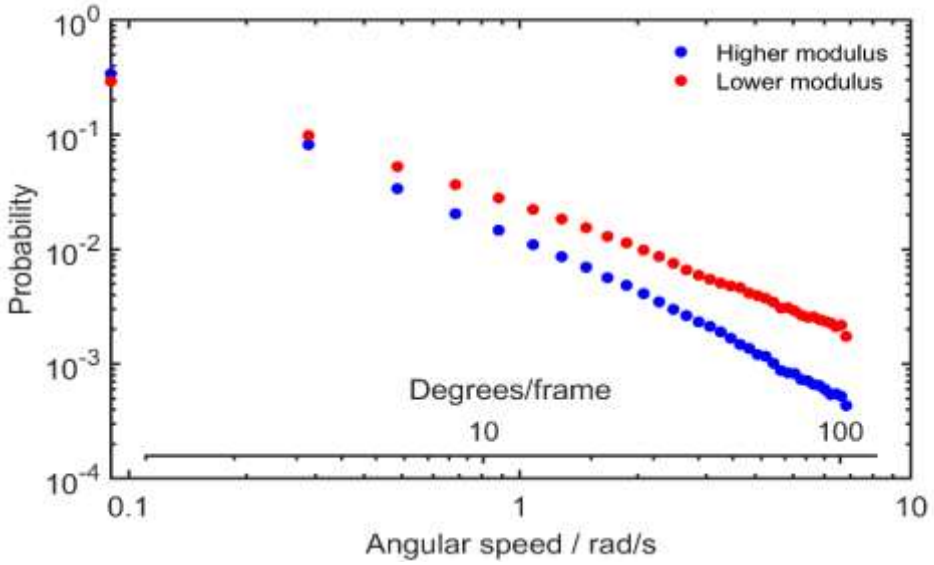


Fig. 11. Probability distribution of angular speed for *P. aeruginosa* on higher modulus (6,700 kPa) and lower modulus (69 kPa) substrates. The probability decreases monotonically for both substrates. The probability for all turns greater than about 3^{o} per frame is greater for the lower modulus material.

Whether slingshots occurred on PDMS and their effect on mean speed were also investigated. Data from high framerate (one frame per 0.22 s) experiments designed to resolve the high-speed behavior of the bacteria on the high and low modulus materials were analyzed. The high framerate should resolve individual slingshots. High angular speeds for a small fraction of bacteria are observed, which is consistent with the concept of a slingshot, but it is part of a continuous spectrum of incidents involving fast rotation (Fig. 10). That is, we did not observe a distinct range of angular speeds over which the behavior of the bacteria are markedly different. However, the probability of an event is greater on the lower modulus material for all angles greater than about 0.3 rad/s (corresponding to about 3^{o} per frame). The probability of turns greater than 45^{o} per frame is very low (5% on the lower modulus and 1.5% on the higher modulus) but about 3.5 times greater on low modulus material. Thus, it can be concluded that "slingshots" (here used to designate turns with large angle) occur more commonly on the lower modulus material, which is consistent with prior work. [56] However, the high angular speed events occur so rarely that their impact on the average speed is minimal when averaged over periods of one minute or greater (Fig. 12A).

As an alternate representation, the same information is plotted in Fig. 12B as the MSD as a function of time interval, Δt. The MSD is frequently characterized as a power law function:

$$\mathrm{MSD} = a\,(\Delta t)^n, \qquad (2)$$

where a and n are constants associated with a particular set of experimental conditions. An exponent of $n = 1$ represents a random walk, and $n = 2$ represents straight-line motion. Fits to the data show $n = 1.4$ for the lower modulus material and $n = 1.3$ for the higher modulus material, but a Student's t-test on the two sets of data yield p = 0.33, which we interpret as not resolved a difference in n-values for the two materials.

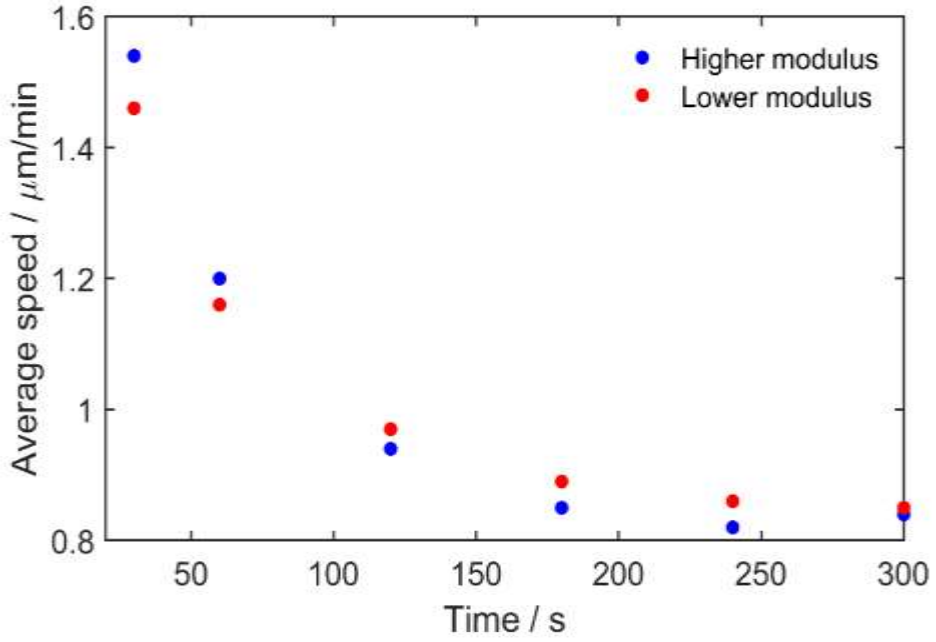


Fig. 12 a. Bacterial speed as a function of time interval. Over times of more than one minute, the average speed is about the same on the more stiff and the less stiff materials.

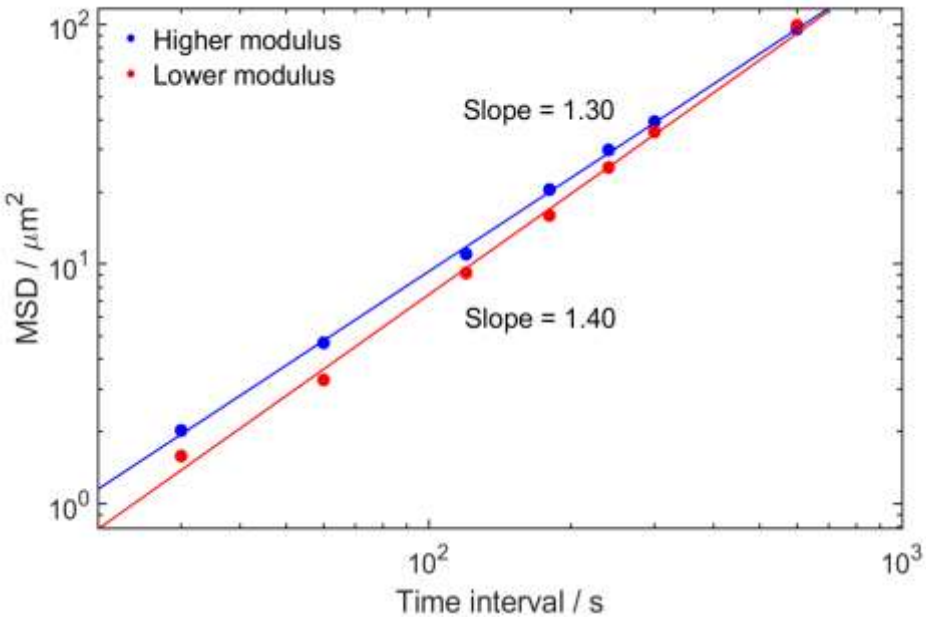


Fig. 12 b. Bacterial MSD as a function of time interval (log-log plot). Points are the average of three experiments; lines are the best fit to Eq. 2 to the average values.

Chapter 4. Discussion

The purpose of this work is to investigate the effect of substrate Young's modulus and surface chemistry on the motile behavior of *P. aeruginosa*, with the aim of finding surfaces or surface properties that could mitigate biofilm formation. Most notably, it was found that the mean speed of *P. aeruginosa* is significantly greater on the hydrophobic surface chemistry.

4.1 Surface chemistry

It was found that *P. aeruginosa* tended to move at a greater average speed on the hydrophobic solid. There are a number of possible reasons for this effect. If the fraction of bacteria in the mode that produces the biggest displacements (crawling) were greater on the hydrophobic solid, then this would go some way toward explaining why the speed was greater on the hydrophobic solid.

It is therefore of interest to know the ratio of modes. To identify the modes of motion, the apparent bacterial aspect ratio was examined. Since walking bacteria have a long axis approximately perpendicular to the surface, which is parallel to the direction of light propagation, they appear to have a low aspect ratio, whereas crawling bacteria have their long axis parallel to the surface plane and therefore appear in the microscopy images with a large aspect ratio. To determine the aspect ratio, an ellipse was fitted to the image of each bacterium and the ratio of the major to the minor axes was calculated. A frequency distribution of aspect ratios, shown in Fig. 13, indicates that there are more low-aspect ratio bacteria on the hydrophobic solid, which is interpret as a greater fraction of walkers on the hydrophobic solid. To enable a quantitative comparison, a cut-off was semi-arbitrarily made at a ratio of 1.25, below which the bacterium is considered to be a walker, and above which a crawler. Table 6 shows the probability of aspect ratio below 1.25 for three repeat measurements: the average is significantly larger for the hydrophobic solid (p = 0.02), which is interpreted as a greater fraction of walkers. This result is not consistent with the

original hypothesis, which was that the higher average speed on the hydrophobic solid might be explained by a lower fraction of walkers compared to the hydrophilic solid.

In previous work, it was found that walkers have lower persistence lengths than crawlers, which should correspond to lower speeds.[32] Figure 14 shows the frequency distribution of speeds, but now with each trajectory divided into walking or crawling modes based on the aspect ratio. There is a higher probability of low speeds for the walkers on both substrates, which is consistent with earlier work.[32] Combining the results of a greater fraction of walkers on the hydrophobic solid, the lower average speed of these walkers, and the overall greater speed of the bacteria on the hydrophobic solid, it is concluded that the bacteria on the hydrophobic solid have greater speed despite the greater fraction of walkers, not because of it.

Table 6. Proportions of bacteria with low aspect ratio ((major axis / minor axis) < 1.25)

	Proportion of bacteria with AR < 1.25	
Replicate	**Hydrophilic**	**Hydrophobic**
1	0.03	0.08
2	0.00	0.06
3	0.01	0.04
Average	0.01	0.06

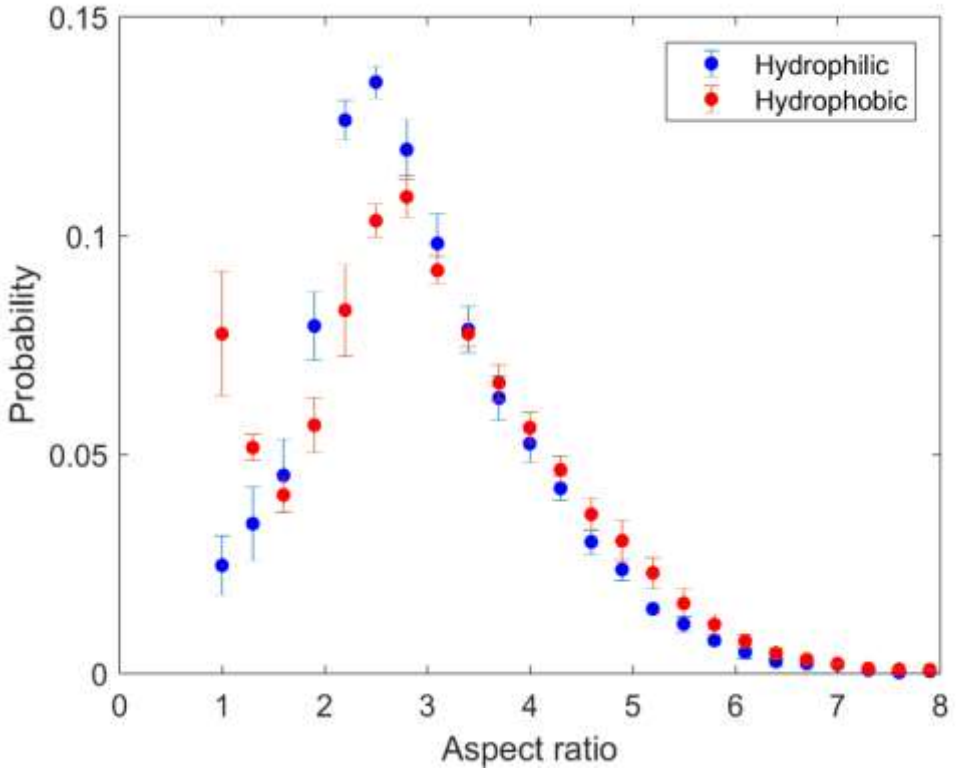


Fig. 13. Probability distribution of aspect ratio (major/minor axes) for *P. aeruginosa* on hydrophilic and hydrophobic substrates. Error bars represent $\pm$ one standard error. A low aspect ratio is interpreted as a walker; the data indicate a greater fraction of walkers on the hydrophobic solid. There is a large range of aspect ratios of crawlers because the bacteria have a length that depends on the stage of the division cycle. Aspect ratios greater than about four indicate possible segmentation errors where the cells have probably already divided, but were identified as a single cell.

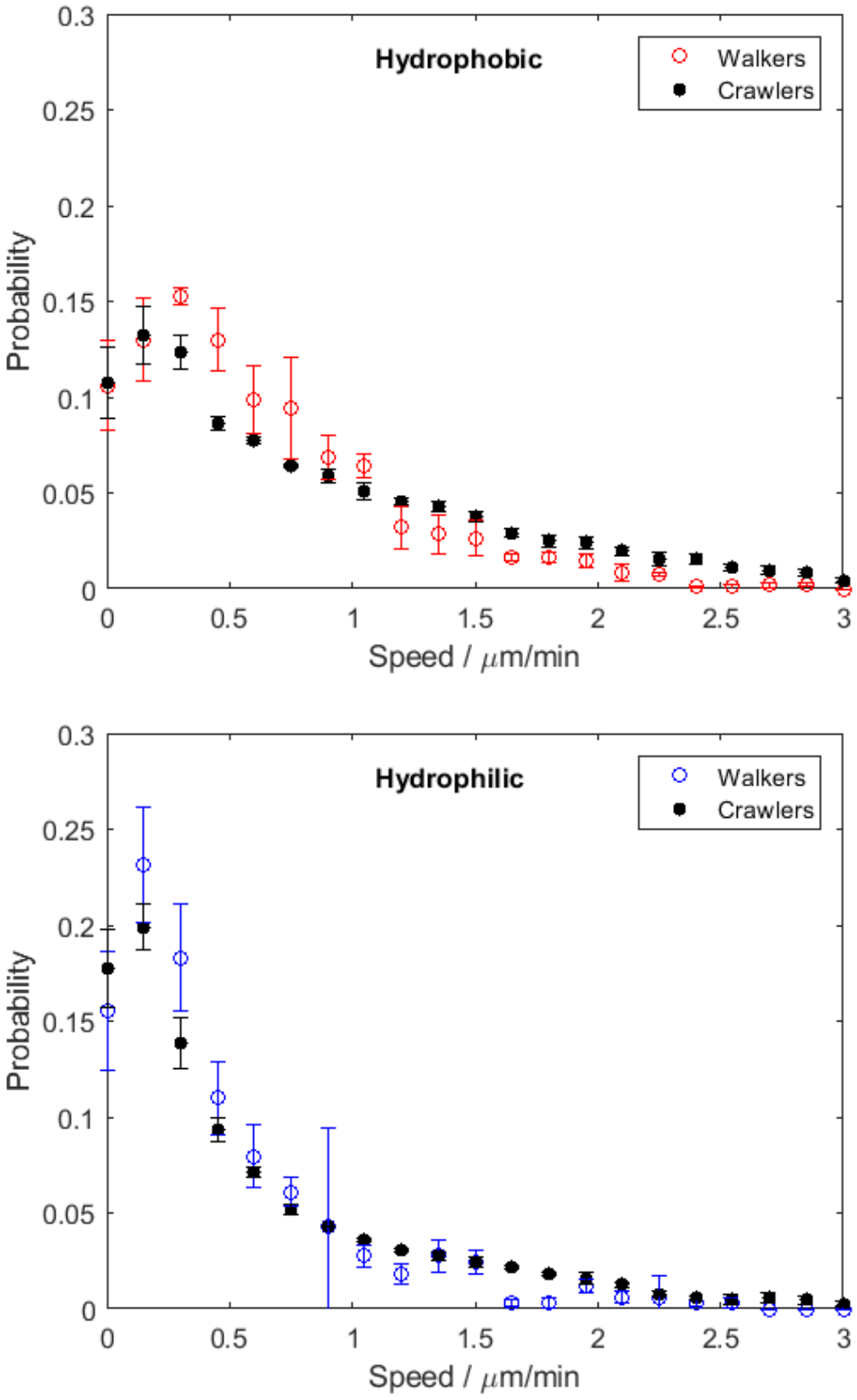


Fig. 14. Relative frequency distributions of bacterial speeds, split into walkers and crawlers (as determined by aspect ratio) for each substrate. Walkers more frequently have lower speeds, while crawlers have a higher frequency of higher speeds. This is especially true for the hydrophobic substrate.

Table 7 shows that large turns were less frequent on the hydrophilic solid. Thus, the slower speed on the hydrophilic surface is not attributable to a greater probability of large-angle turns. The surface wettability significantly affects surface motility – the hydrophilic solid has a lower fraction of bacteria in the walking mode and a lower frequency of rapid changes in direction. These differences should produce higher linear speeds, but rather the speeds are lower on the hydrophilic solid. Therefore, the effect on average speed mainly has to do with the greater fraction of cells that remain almost stationary on the hydrophilic solid.

Large, sudden changes in orientation should decrease the average linear speed of a bacterium. Because the course of a turn was not resolved, it was assumed that all turns had a magnitude less than 180°, and positive and negative frequencies of the same amplitude were lumped together. Changes near 180° were excluded because it is not possible to distinguish one end of the bacterium from another and thus, for example, 5° turns and –175° turns appear the same.

In this work, a large change in angle is regarded as one in the range of 10 – 40% of a complete circle.

Table 7. Proportions of bacteria that make a turn of 10 – 40% of a complete circle in 30 sec.

	Proportion of bacteria that make a 10-40% turn	
Replicate	**Hydrophilic**	**Hydrophobic**
1	0.06	0.08
2	0.06	0.10
3	0.05	0.07
Average	0.06	0.08

With the information at hand, it is only possible to speculate as to the mechanism of why there are more stationary bacteria on the hydrophilic solid. There may be a bacterial surface sensing element (such as a pilus) that detects the surface chemistry and biases a biochemical pathway that suppresses motility. On the other hand, it may simply be a matter of the adhesion energy. The measurement of the aspect ratio provides some insight into this possibility. Recall that the aspect ratio is used as a proxy for the orientation of each bacterium. From Table 6 it can be concluded that the probability of an orientation parallel to the surface normal is six times smaller on the hydrophilic solid. Because the bacterium has a rod-like shape, this is consistent with a greater adhesion of the body of the cell to the hydrophilic solid. If the adhesion is stronger, then the bacterium is more likely to be immobile, as observed.

4.2 Modulus

Although the Young's modulus did affect the frequency of high-speed turns, it had little effect on the average motion over a period of minutes. These results suggest that changing the modulus is not a promising avenue for retarding the movement of *P. aeruginosa*. Materials that have an even lower stiffness than those considered here (<25 kPa) are usually gels or similar materials that are not suitable for use in tubing, etc., in medical devices. In addition, such low modulus materials are often porous or otherwise inhomogeneous so it is not clear that changes in motility could be directly attributable to the modulus.

Recent work by Sabass et al.[57] describes measurement of the force applied by the type IV pilus during twitching motility of *Myxococcus xanthus* by measuring the deformation of a support. To resolve the movement of the support they used a highly porous gel of polyacrylamide (PAA) with a shear modulus of 121 Pa. Even for this extremely low modulus, which is only 5% of the lowest modulus examined here, the deformation of the support was only about 100 nm. This is <10% of the typical retraction length of a pilus.[30]

For these reasons, it seems unlikely that deformation of a solid would have a significant effect on forward motion mediated by type IV pili for the much stiffer materials that were used here or in indwelling devices such as catheters. Although the tiny deformations may not directly affect forward motion, this does not rule out the possibility that the bacterium has some mechanosensing element that makes the cell biochemically responsive to surface stiffness. Further exploration of this subject is available in the appendix.

4.3 Clinical application

The results of this work could potentially find applications in clinical settings. For example, in one survey of more than 12,000 patients, the authors found that more than half of hospitalized patients with healthcare-acquired infections had at least one catheter in place.[6] This compares with 17% of patients overall (both those with and those without healthcare-acquired infections) who had at least one catheter in place.

By controlling the wettability of the exterior of a catheter, it may be possible to decrease the bacterial motility or adhesion energy that contributes to biofilm formation and ultimately to decrease patient infection rates.

Another potential application involves developing coatings with specific chemistries for mechanical heart valves. Once a biofilm forms in or on a mechanical heart valve, in most cases it cannot be permanently remediated.[5] By inhibiting or slowing biofilm formation, perhaps a patient's immune system would have more time to combat a developing biofilm.

It is important to note, however, that bacterial responses depend on many factors, such as the bacterial strain and nutritional conditions, so studies under the appropriate clinical conditions would be required for clinical conclusions.

Chapter 5. Conclusions

The average distance moved *by Pseudomonas aeruginosa* is reduced on surfaces that have been treated with oxygen plasma. The oxygen plasma treatment reduces the contact angle from 100^{o} to 0^{o} (makes the solid much more hydrophilic). The mean speed of *P. aeruginosa* is approximately 40% lower on hydrophilic PDMS. This is despite the observation that on the hydrophilic solid, the bacteria less frequently make large-amplitude turns, and there is a lower fraction of cells in the lower persistence length walking mode. The reason for the reduced average speed is that there is a greater fraction of cells on the hydrophilic surface that are nearly immobile. The bacteria show recognition of the surface chemistry in two ways: (1) by more frequently remaining immobile over long periods of time and (2) by changing their orientation relative to the surface normal. These two effects may have a common origin in a stronger adhesion energy of the body of the cell to the hydrophilic solid. It is interesting that the recognition of surface chemistry occurs despite the presence of a rather complex mixture of chemicals in TSB, the growth medium used here. The bacteria may be sensing the solid substrate, or the various substances adhered to the substrate. In contrast, the Young's modulus of the solid has little effect on the average speed. Because bacterial behavior may depend on environmental conditions, these conclusions cannot immediately be generalized to behavior of other bacterial strains or other conditions of nutrition, etc., but do illustrate that surface chemistry can affect surface motility.

Chapter 6. Future Work

In this work, the surface chemistries under consideration span a wide range of hydrophilicities. No attempt has been made to investigate other facets of surface chemistry, such as surface energy or the adsorption of various species to the substrate, which may yield different results. In addition to probing the motile behavior of bacteria, such investigations might yield mechanistic insight into the behavior and properties of type IV pili.

One of the primary conclusions of this work is that bacterial motility can be influenced by the surface chemistry of the underlying substrate. This is despite the presence of a complex liquid medium, some of the components of which no doubt adhere to the surface and partially obscure the surface chemistry. It may be interesting to consider the role of the medium in screening the interactions between a bacterium and a surface. In particular, for growth media that contain substances strongly attracted to the surface, it might be reasonable to expect that the effect of surface properties on motile behavior would be reduced. That is, the more obscured the surface chemistry is by adsorbed species, the less impact it may have on motility. Such an investigation might be challenging because altering the composition of the growth medium could reasonably be expected to change the behavior of the bacteria, regardless of the substrate. If defined (and perhaps minimal) growth media were utilized, it might enable sufficiently precise control over adhesion of material to the substrate without metabolically altering bacterial behavior.

Finally, it may be worthwhile to conduct more direct mechanistic investigations into the interactions between the bacterial membrane, the type IV pilus, and the substrate. In particular, the use of fluorescent tagging to obtain clear images of the pilus might offer one avenue for determine how it is that surface chemistry can mediate motility. This may help illuminate outstanding variables such as:

1. Does *P. aeruginosa* produce more pili on surfaces with specific chemistries?

2. Does surface chemistry influence the distribution of pili relative to the bacterium (ex. more pili at one pole than at the other)?
3. Do pili release more frequently from surfaces with specific chemistries? This could have an impact on rotational motion as well as linear motion.

These questions would be difficult to answer without direct imaging of the pili. Not only would such imaging allow for the investigation of additional variables, it would also allow for the correlation of long-time (periods of minutes or hours) motile behavior with short-time (periods of seconds) behavior of the pili.

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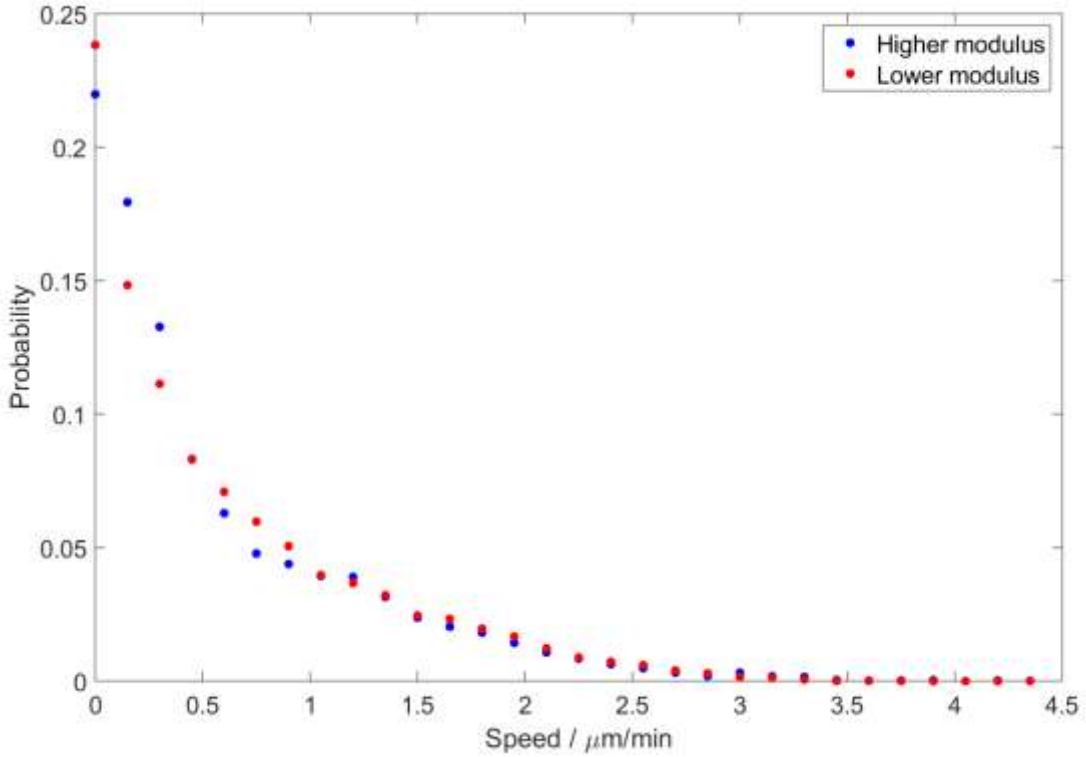
Appendix A



Probability / (min/µm) of *P. aeruginosa* speeds over a period of 5 min, compared for higher (6,700 kPa) and lower (69 kPa) Young's modulus substrates. Data collected over 60 min.

Note that the curves do not appear to be significantly different from one another.

Appendix B

The following consists of a report written on the subject of finite element modeling of the pilus-substrate system. The purpose of this investigation was to determine whether it was reasonable to believe that surface modulus could affect bacterial motility.

Abaqus Simulations of Pilus-Substrate Interactions

Background

A pilus advances a bacterium by attaching at a point on the solid and then shortening the length between that point and the bacterium. If the attachment point moves toward the bacterium on the time scale and length scale of the pilus movement, then the net movement of the bacterium is appreciably reduced. If the energy of deformation of the solid is significant compared to the total energy (for both movement and deformation of the solid combined) expended by the bacterium, then the bacterium is wasting an appreciable amount of energy.

Objectives

1. Does the surface deform at a significant rate?
2. Does surface deformation waste a significant amount of energy?

In more detail:

1. The strain of the substrate as a function of time. Strain may increase such that the deformation of the solid takes place on the same order of length as the pilus itself. If this happens over a reasonable timescale, then strain within the solid becomes a relevant quantity governing motility of the bacterium.
2. The total energy imparted by the pilus into the solid. If this energy is of the order of the kinetic energy of a bacterium undergoing motility, then stress-strain energy may be a limiting factor associated with motility.
    a. A sub-objective has to do with the power output of the molecular motor powering pilus-mediated motility. This quantity can also be related to the total metabolic output of a bacterium.

Metrics

Each of the above objectives is associated with a unique metric:

1. A pilus is in contact with a surface for a finite length of time. Over this time, the pilus causes a deformation of the substrate. We want to know, at the conclusion of this interval of contact, the total (final) deformation, *a*, as measured by strain.
    a. Note that we are really interested in a quantity related to *a*. That is, we want to know the linear deformation collinear with the pilus.
2. The total stress-strain energy of the substrate at the conclusion of the interval of contact.
    a. This metric is complicated by the fact that the motor can only sustain a certain maximum power output.

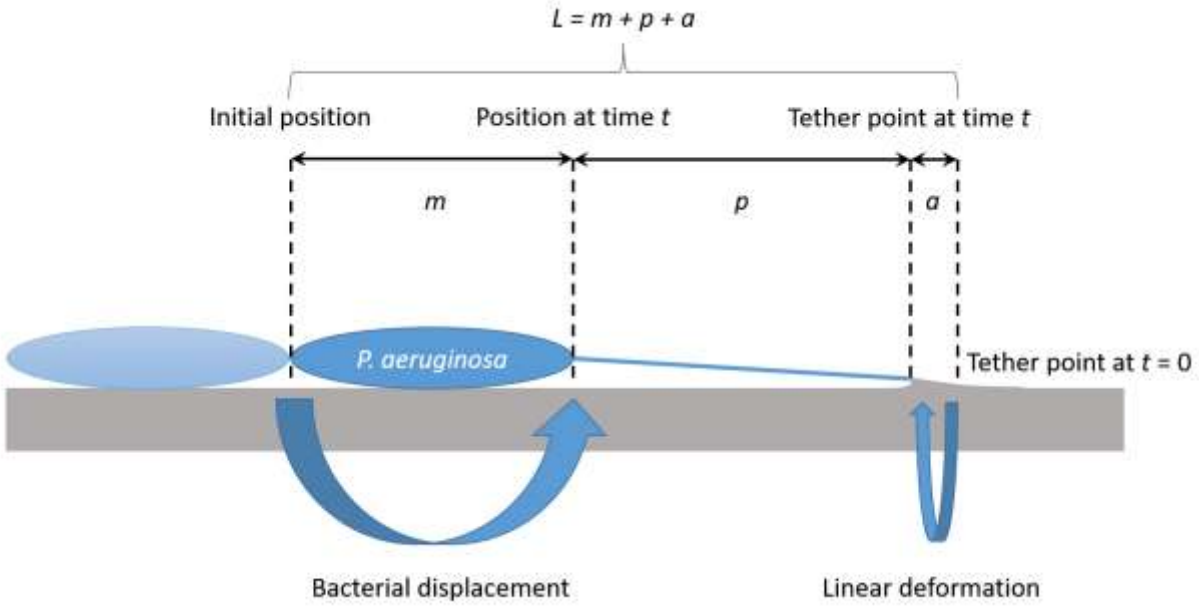
Equations

L the (constant) starting distance from the point of attachment of the pilus to the bacterium.

a the deformation distance of the solid (could be at both ends of the pilus but we simplify having it at one end). As noted above, we really want the deformation collinear with the pilus, which is a quantity closely related to *a*.

p the length of the pilus (assumed rigid).

M the distance of movement of the pilus, and therefore, of the bacterium.



$$L = m + p + a$$

$$\frac{dL}{dt} = 0 = \frac{dm}{dt} + \frac{dp}{dt} + \frac{da}{dt}$$

The velocity of the bacterium is given by:

$$\frac{dm}{dt} = -\frac{dp}{dt} - \frac{da}{dt}$$

The pilus shortens with time so $\frac{dp}{dt}$ is negative, and the deformation grows with time, so $\frac{da}{dt}$ is positive. Therefore, any finite value of $\frac{da}{dt}$ reduces the velocity of the bacterium.

Materials Data

I ran the samples through a simple parallel plate rheometer to measure the Young's modulus of each sample. I find that the thermally aged 184 substrates have a Young's modulus of approximately 250 kPa. The aged 8_1 substrates have a Young's modulus of approximately 25 kPa. I have used these moduli for the simulations. PDMS has a Poisson's ratio of very nearly 0.5. This presents some computational difficulties; consequently, I have used a Poisson's ratio of 0.495 for the simulations.

One-cylinder test

A simple model of pilus motion is that the pilus is an infinitely rigid cylinder and we consider only a cylindrical element of the solid with finite modulus and a Poisson's ratio of zero.

At mechanical equilibrium:

$\sigma = \epsilon E$, where E represents the Young's modulus of the material.

So we predict that strain will be inversely proportional to Young's modulus, for a fixed stress.

Two-cylinder test

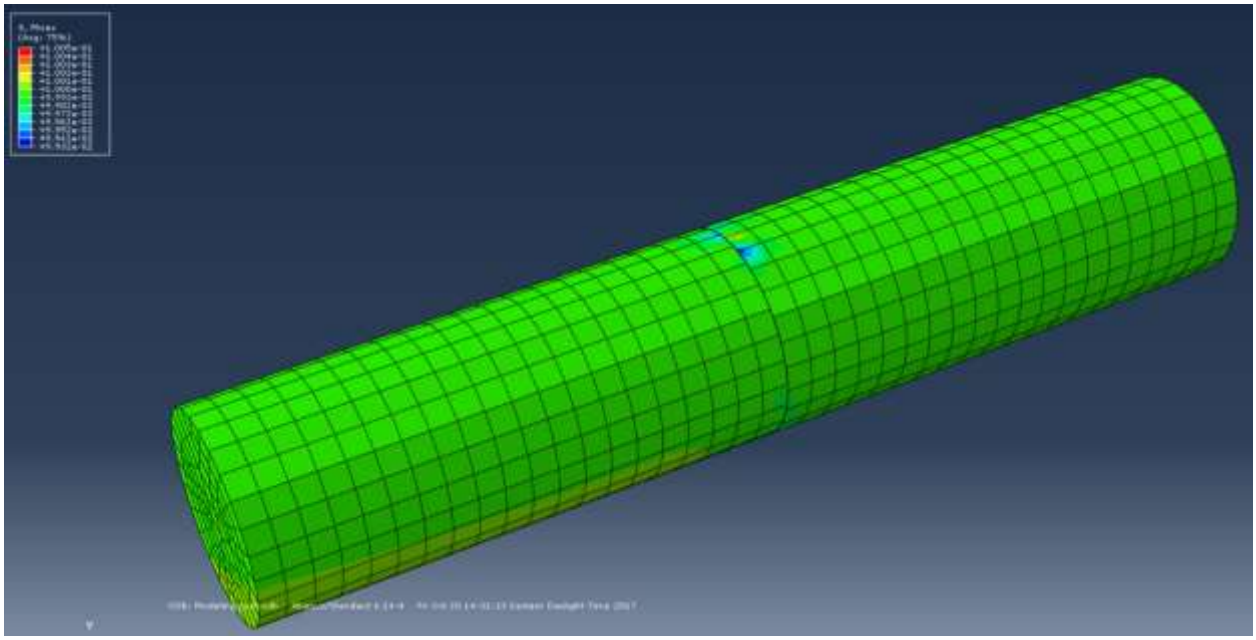
A simple model of pilus motion is that the pilus is a cylinder and we consider only a cylindrical element of the solid.

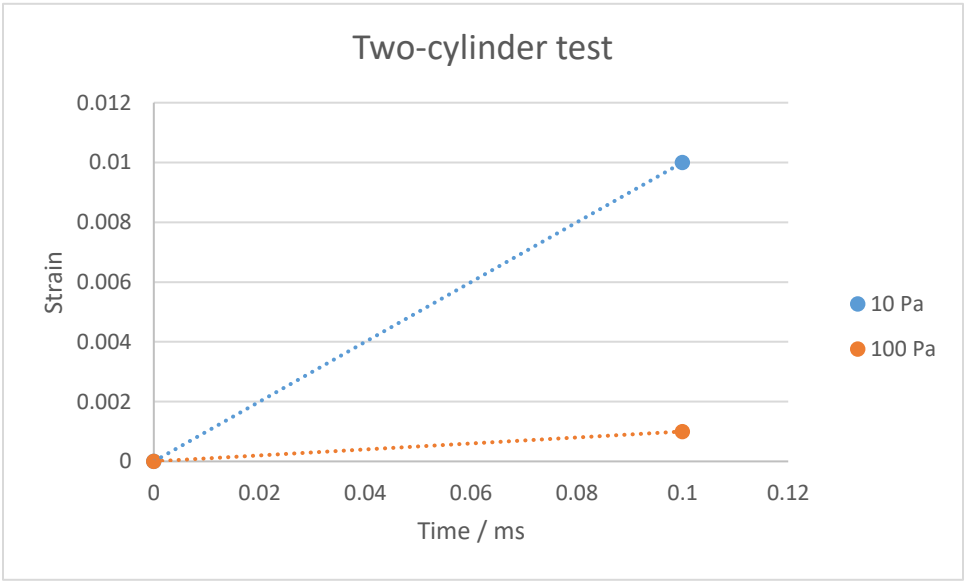
At mechanical equilibrium:

$\sigma = \epsilon_1 E_1 = \epsilon_2 E_2$

Therefore, $\epsilon_2 = \epsilon_1 \frac{E_1}{E_2}$

I reproduced this phenomenon using Abaqus 6.14-4. A cylinder with a high (ten times higher) Young's modulus exhibited only one tenth of the strain under a fixed load.





More realistic models

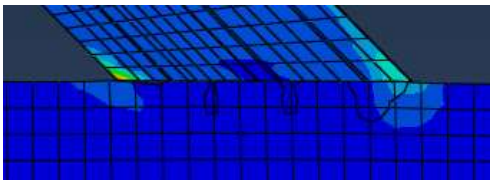
As discovered by Volkmann et al. by way of cryo-electron microscopy, type IV pili have a radius of approximately 3 nm.[58] Maier et al. report that type IV pilus motors are capable of exerting forces in the range of 100 – 200 pN.[59] Therefore, a reasonable upper estimate for the stress exerted during retraction by a type IV pilus on a substrate is:

$$\frac{200 * 10^{-12}\ N}{\pi(3 * 10^{-9}\ m)^2} = \mathbf{7 * 10^6 \frac{N}{m^2}}$$

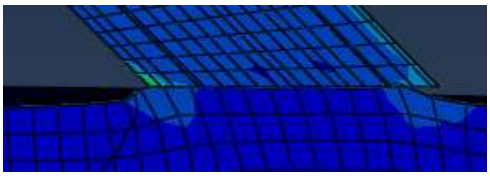
In the below models, I have attempted to match the maximum stress exerted by the pilT protein. For purposes of the simulation, I manage to achieve approximately 15% of this total (1 MPa).

Features: pilus at a 45 degree angle to substrate, arbitrary radius (2 arbitrary units), 0.001 arbitrary time units (1 ms).

Young's modulus = 250 kPa



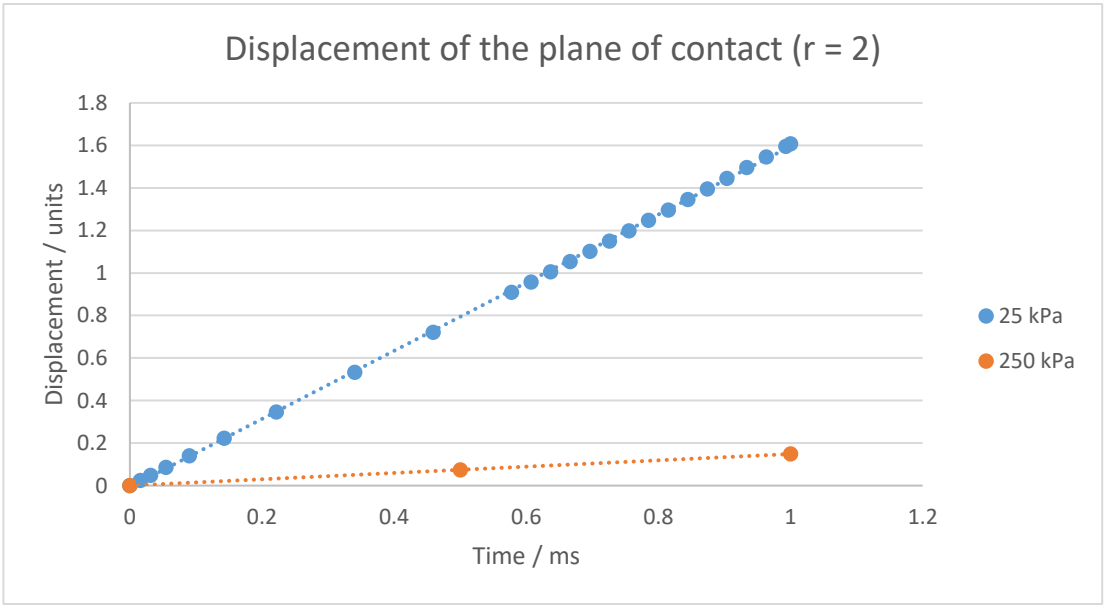
Young's modulus = 25 kPa



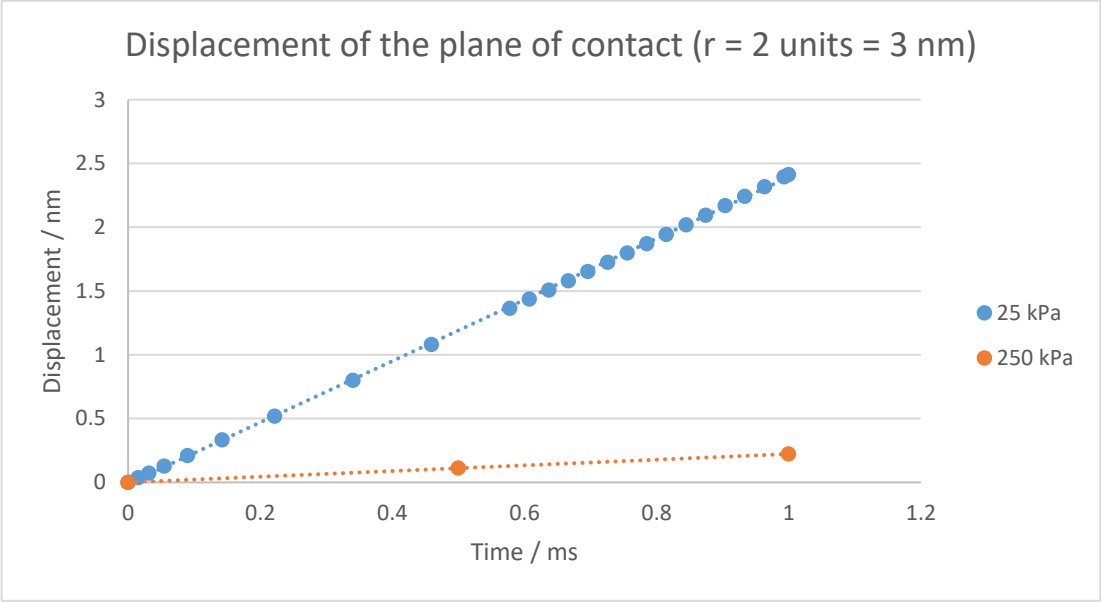
Note that the 250 kPa substrate deforms only very slightly, whereas the 25 kPa substrate exhibits substantial deformation after 1 ms.

The below calculations summarize the average speeds of the substrate (in the region of contact between pilus and substrate) over the 1 ms interval in the simulation. The speeds may not be sustainable over longer time intervals, as the pilus and the substrate must eventually achieve mechanical equilibrium.

Arbitrary units (radius = 2 units)



Arbitrary units converted to nm (2 units = 3 nm):

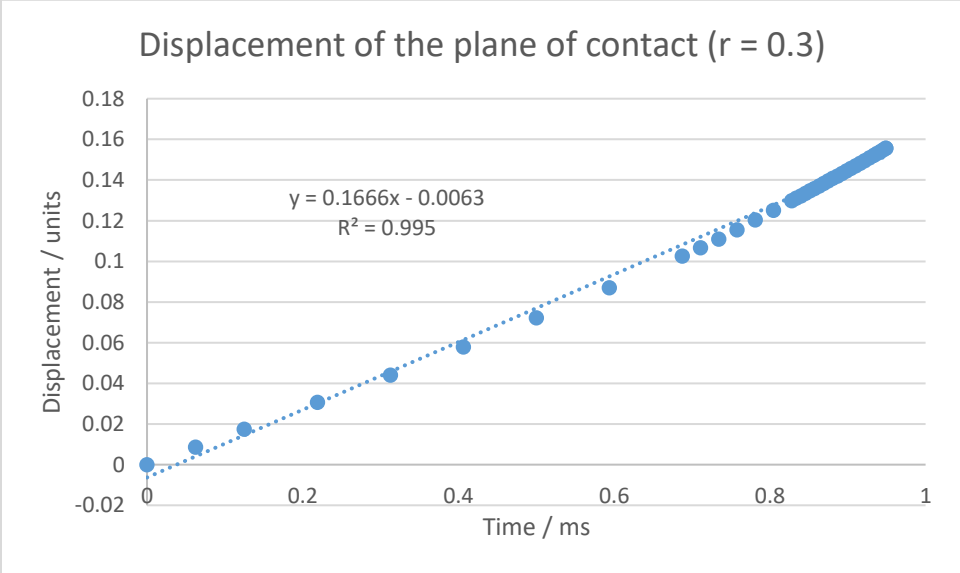


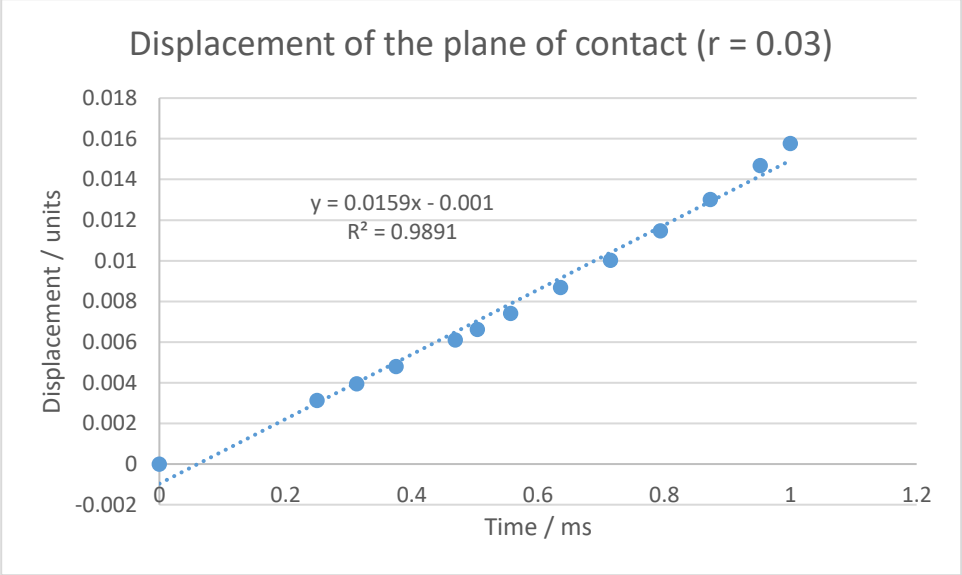
25 kPa: (1.60802 units)/(1.0*10^{-3} s) = 1608.02 units/s = **2400 nm/s = 2.4 µm/s**

250 kPa: (0.148433 units)/(1.0*10^{-3} s) = 148.433 units/s = **220 nm/s = 0.22 µm/s**

Note that these speeds are generally on the same order as the speed of a pilus. If these speeds were sustained for significantly longer than 1 ms, they might affect the motile behavior of a bacterium. We anticipate that at some point, mechanical equilibrium with the substrate will result in a speed (of the plane of contact) of zero. Furthermore, in our simulations we do not reach a large enough timespan to approach mechanical equilibrium.

The following plots show displacement of the plane of contact for smaller radii (25 kPa substrate).





In each of these simulations, the element sizes have been kept approximately constant relative to the radius of the pilus. If we are interested in making the simulations more exactly comparable, it is possible to modify the models such that the element sizes are held more exactly constant relative to the radius.

Power output of pilT

The hexameric pilT protein is responsible for the assembly and retraction of the pilus. Pilin units are added to the pilus, and subsequently the pilT protein conducts hydrolysis of ATP. This results in a conformational change in pilT that forces the pilus outward, resulting in extension. The retraction process is broadly symmetric. That is, the pilus is retracted and depolymerized due to action of the pilT protein associated with conformational changes brought on by ATP hydrolysis.

I propose the following estimate for the power output of the pilT protein:

$$\frac{dE}{dt} = \frac{\Delta G_m}{N_A} * \frac{dP}{dt}$$

Where:

$\frac{dE}{dt}$ power output by the pilT protein

ΔG_m molar Gibbs energy of ATP hydrolysis

N_A Avogadro's number

$\frac{dP}{dt}$ rate of addition of pilin units to the pilus [(pilin units)/s]

Using the following quantities and estimates:

$$\Delta G_m = 50\frac{kJ}{mol} \qquad N_A = 6.022 * 10^{23}\frac{molecules}{mol} \qquad \frac{dP}{dt} = 10{,}000\ \frac{pilin\ units}{s}$$

$$\frac{dE}{dt} = \left(\frac{50 * 10^3\frac{J}{mol}}{6.022 * 10^{23}\frac{molecules}{mol}}\right)\left(1\frac{molecule\ ATP}{pilin\ unit\ added}\right)\left(10{,}000\ \frac{pilin\ units}{s}\right) = \mathbf{8.3 * 10^{-16}\frac{J}{s}}$$

The estimate $\Delta G_m = -50\frac{kJ}{mol}$ is commonly used in the biological literature.[60] Although the physical chemistry of our system could be somewhat unusual, I have no reason to believe that is the case. The estimate $\frac{dP}{dt} = 10{,}000\ \frac{pilin\ units}{s}$ is slightly more suspect, and is made upon observation that type IV pili may be multiple microns in length, and that they may take one or more seconds to fully extend.[61] Considering that a pilin unit is approximately 0.1 nm in length, the rate of pilin addition is likely on the order of 10,000 units per second.[30]