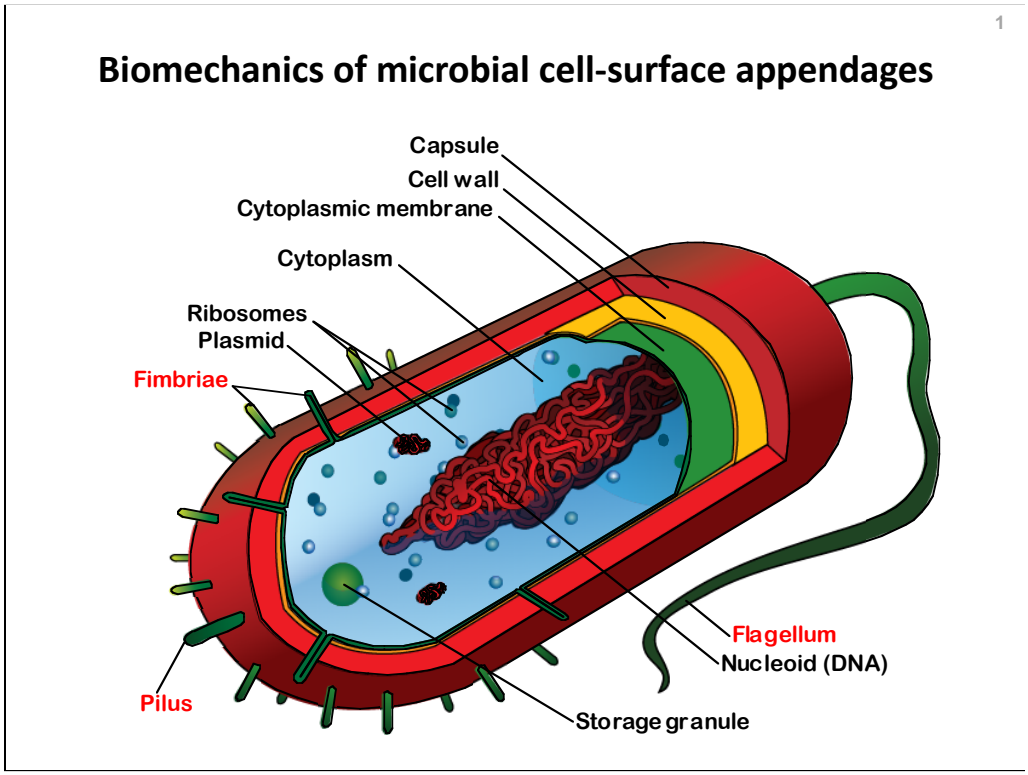


Source: http://www.microbiologynotes.com/differences-between-fimbriae-and-pili/

Pili (singular: pilus) and **fimbriae** (singular: fimbria) are filamentous structures composed of proteins that extend from the surface of a cell. They can have many functions. Fimbriae are found in Gram-negative and Gram-positive bacteria but they are shorter in length than pili. Pili are longer and more complex than fimbriae and there are typically only a few pili per cell. In this lecture we will focus mainly on pili because they are more interesting than fimbriae from a biomechanical perspective.

Some of the differences between fimbriae and pili are as follows:

(1) Definition. Fimbriae are tiny bristle-like fibers arising from the surface of bacterial cells. Pili are hair-like microfibers that are thick tubular structure made up of pilin.

(2) Length. Fimbriae are shorter than pili. Pili are longer than fimbriae.

(3) Diameter. Fimbriae are thinner than pili. Pili are thicker than fimbriae.

(4) Number. Number of fimbriae: 200-400 per cell. Number of pili: 1-10 per cell.

(5) Proteins. Fimbriae are made of of **fimbrillin** protein. Pili are made of **pilin** protein.

(6) Rigidity. Fimbriae are less rigid than pili. Pili are more rigid than fimbriae.

(7) Distribution. Fimbriae are found in both Gram-positive and Gram-negative bacteria. Pili are found in both Gram-negative and Gram-positive bacteria but they are more diverse and widespread in Gram-negative bacteria.

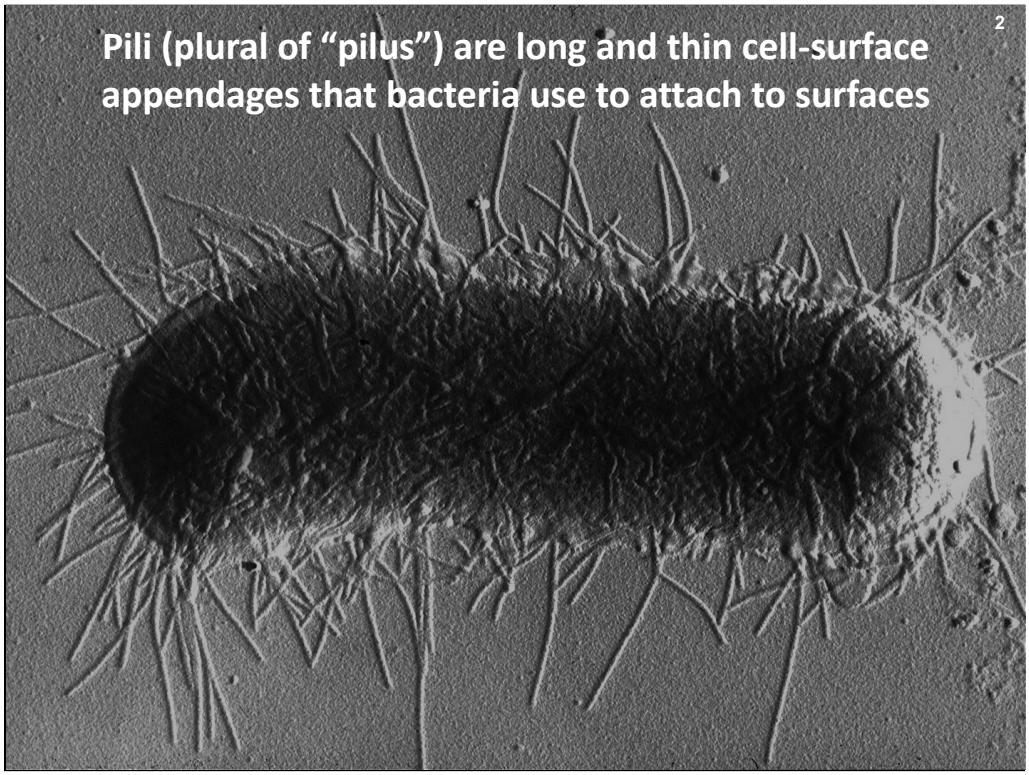
(8) Formation. The genes encoding fimbriae and pili can be found in the chromosome or on extrachromosomal plasmids.

(9) Function. Fimbriae and pili both mediate cell-to-surface attachment and cell-to-cell attachment. Pili also mediate bacterial conjugation (gene transfer between bacterial cells).

(10) Motility. Fimbriae do not function in active motility. Type IV pili drive “twitching motility”.

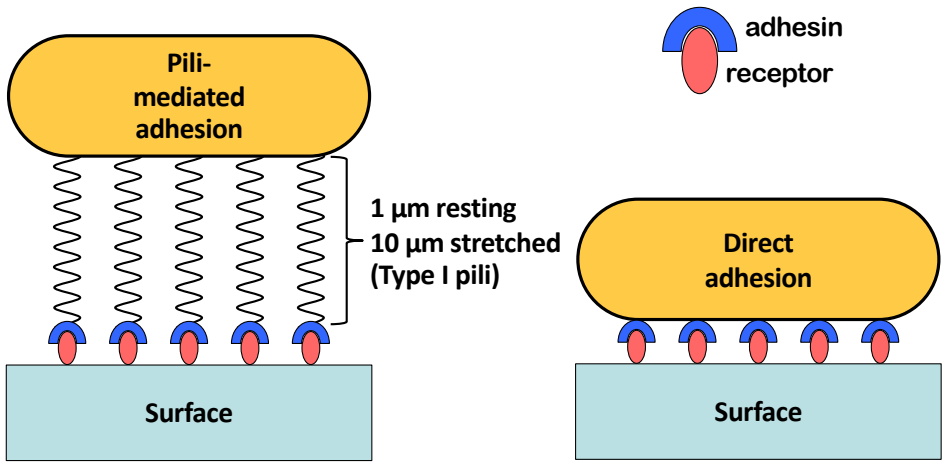
(11) Receptors. Fimbriae do not serve as receptors for bacteriophages (viruses that infect bacteria). Pili serve as receptors for some bacteriophages.

(12) Secretion. Fimbriae do not function as secretion systems for other proteins. Pili do (sometimes) serve as secretion systems for other proteins (e.g., Type III secretion pili, Type IV secretion pili).

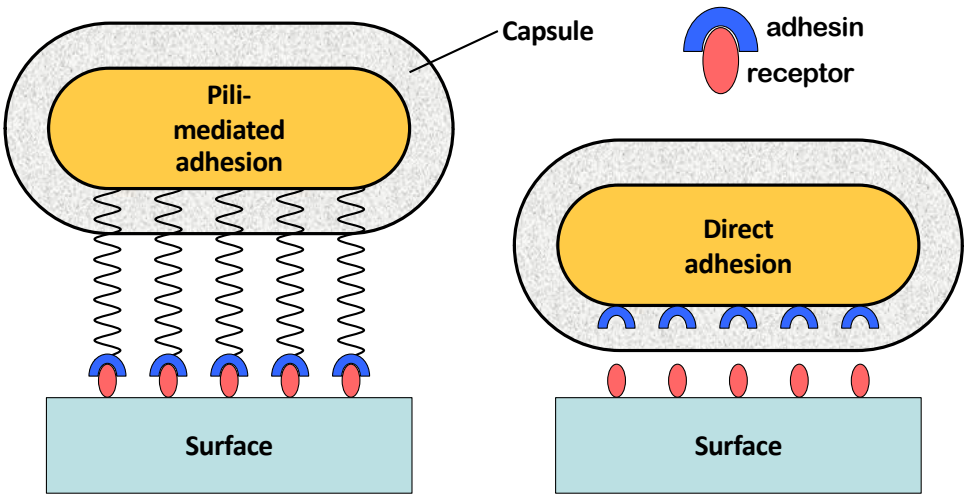


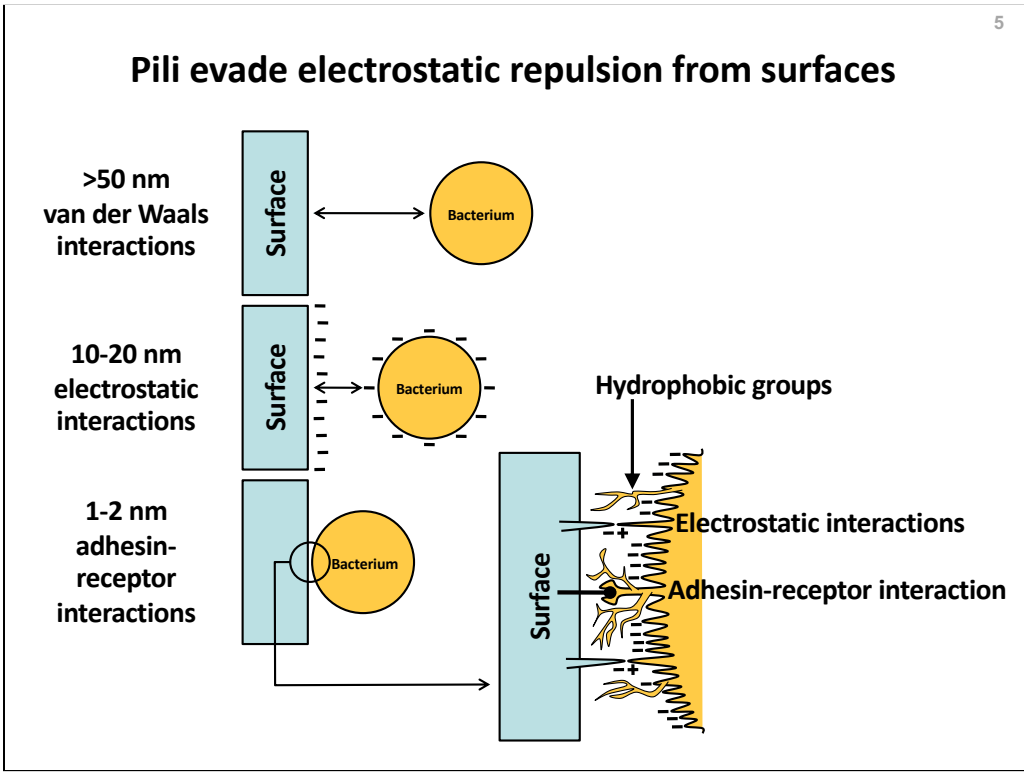
Pilus is singular. Pili is plural.

Pili are elongated and stretchable helices that mediate the attachment of bacteria to surfaces



Pili reach beyond the capsule barrier to mediate attachment of bacteria to surfaces at a distance





Source: Busscher HI, Weerkamp AH (1987) Specific and non-specific interactions in bacterial adhesion to solid substrata. *FEMS Microbiol Rev* 46: 165-173 doi: 10.1111/j.1574-6968.1987.tb02457.x.

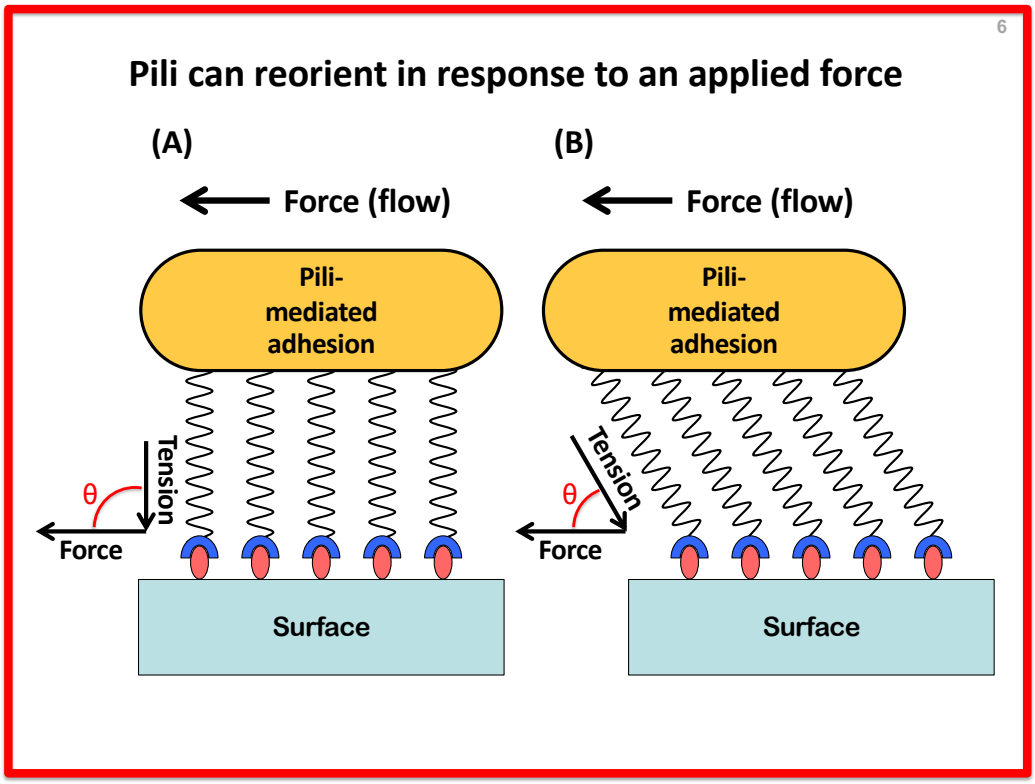
Figure 2. Schematic representation of interactions involved in bacterial adhesion to solid substrates. Three distinct interaction regions are distinguished:

1. Separation distances > 50 nm, at which only **van der Waals forces** operate. These are weak electrostatic attractive or repulsive forces between uncharged molecules arising from the interaction of permanent or transient electric dipole moments. Non-specific, macroscopic cell surface properties play the dominant role in this stage of adhesion.

2. Separation distances between 10 and 20 nm. Additional **electrostatic repulsion** between charged molecules is now becoming active, resulting in a reversible secondary minimum adhesion. The surface of mammalian cells tends to me negatively charged (e.g., due to cell-surface sialic acid). The surface of bacterial cells also tends to me negatively charged (e.g., due to cell-surface lipopolysaccharide).

3. Separation distance 1-2 nm, only at very small separation distances can specific **receptor-ligand interactions** take place, provided the organism is capable of extruding adhesion probes and hydrophobic groups are available to dehydrate the surfaces, facilitating direct contact.

By placing adhesins at the tips of very long pili, adhesins (on the bacteria) can engage with receptors (on the surface) at a distance much larger than the distance over which these repulsive forces can act. For example, type IV pili, which we'll talk about later in this lecture, can be up to 4 microns long. This allows adhesins to bind directly to their receptors while keeping the bacterial cell at a distance well beyond the range where repulsive van der Waals forces or electrostatic forces can interfere.



Imagine the applied force (for example, due to fluid flow) is the same in (A) and (B). The corresponding "equal and opposite" tension in the pili would be:

A. Higher in (A) than in (B).

B. Higher in (B) than in (A).

C. Equal in (A) and (B).

Answer: A. Higher in (A) than in (B). Can you explain why? Can you think of a similar example from the Week_02 lecture?

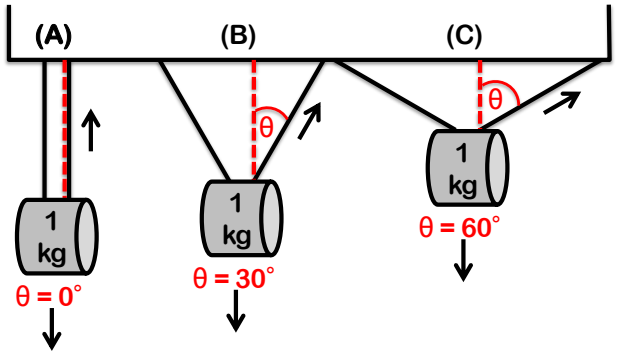
Imagine a 1-kg weight suspended from cables, as shown in the diagram. The tension in the cables would be:

A. Highest in (A).

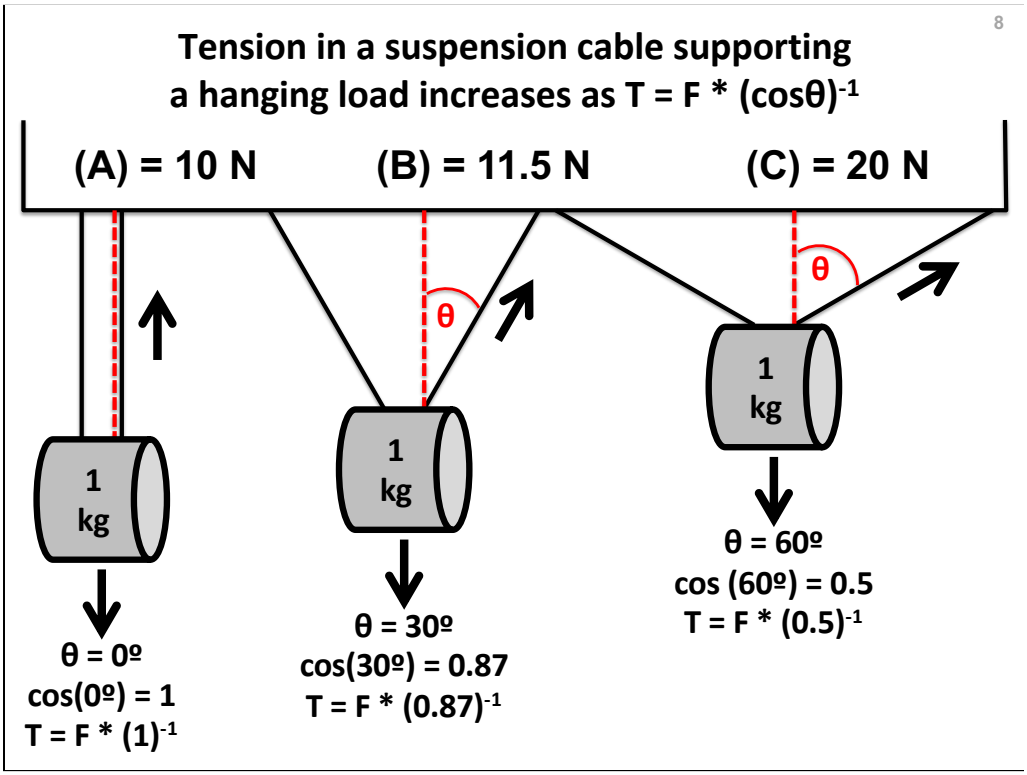
B. Highest in (B).

C. Highest in (C).

D. Equal in (A), (B), (C).



Answer: (C)



Newton's Third Law of Motion: For every action, there is an equal and opposite reaction. In the example shown on this slide, the force exerted by gravity (force = mass * acceleration = kg * 10 * m * s^{-2}) is directed downwards. This downwards force must be balanced by tension in the suspension cable exerting an equal and opposite force upwards. If the cable is perfectly vertical (the force vector angle θ = 0 degrees), then all of the tension in the cable is directed in the "useful" direction (upwards).

In example (a), T = (1 kg) * (10 * m * s^{-2}) * (cos0 degrees)$^{-1}$ = **10 N**

As the angle of the cable relative to the upwards (vertical) direction increases from θ = 0 degrees to θ = 30 degrees the useful (vertical) component of the tension gets smaller and the useless (horizontal) component of the tension gets bigger. Consequently the total tension in the cable must increase in order to provide a sufficient upwards-directed tension to balance the load:

In example (b), T = (1 kg) * (10 * m * s^{-2}) * (cos30 degrees)$^{-1}$ = **11.5 N**

As the angle of the cable relative to the vertical increases from θ = 30 degrees to θ = 60 degrees the useful (vertical) component of the tension gets even smaller and the useless (horizontal) component of the tension gets even bigger. Consequently the total tension in the cable must increase in order to provide a sufficient upwards-directed tensions to balance the load:

In example (c), T = (1 kg) * (10 * m * s^{-2}) * (cos60 degrees)$^{-1}$ = **20 N**

Question: what will be the tension in the suspension cable if we pull the cable perfectly horizontal (θ = 90 degrees)?

T = F/(cos 0 degrees) = F/1 (lowest tension possible)

T = F/(cos 15 degrees) = F/0.97

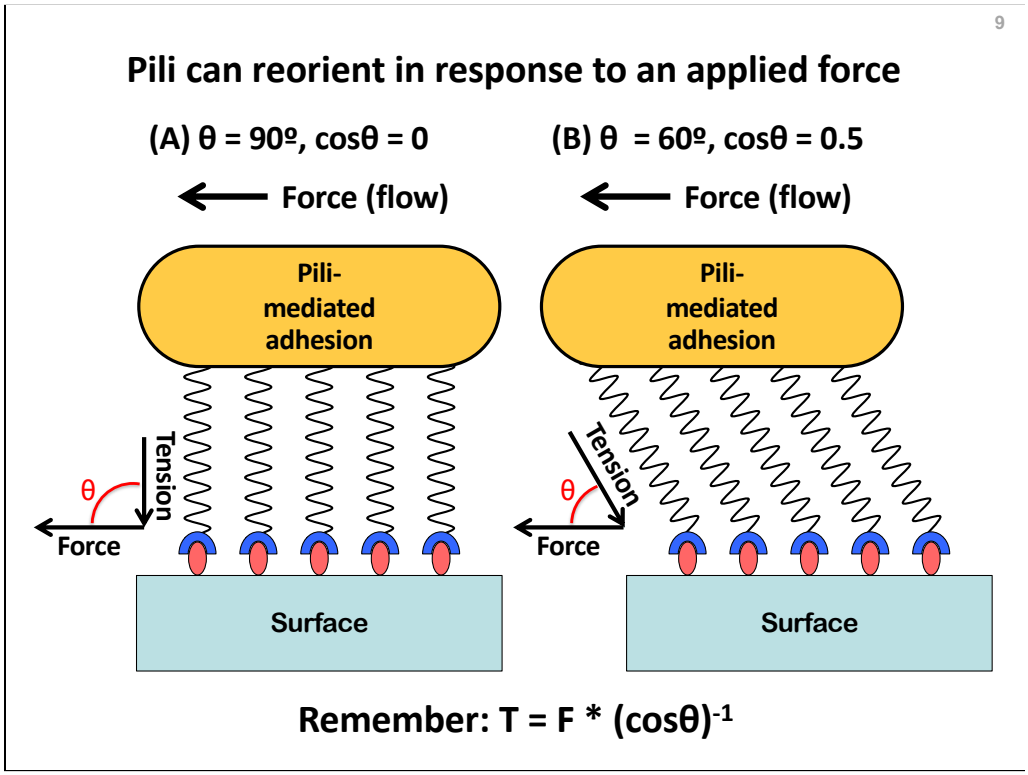
T = F/(cos 30 degrees) = F/0.87

T = F/(cos 45 degrees) = F/0.71

T = F/(cos 60 degrees) = F/0.5

T = F/(cos 75 degrees) = F/0.26

T = F/(cos 90 degrees) = F/0 (infinite tension)



Tension (T)

Force (F)

Force vector angle (cosθ), examples:

T = F/(cos 0 degrees) = F/1

T = F/(cos 15 degrees) = F/0.97

T = F/(cos 30 degrees) = F/0.87

T = F/(cos 45 degrees) = F/0.71

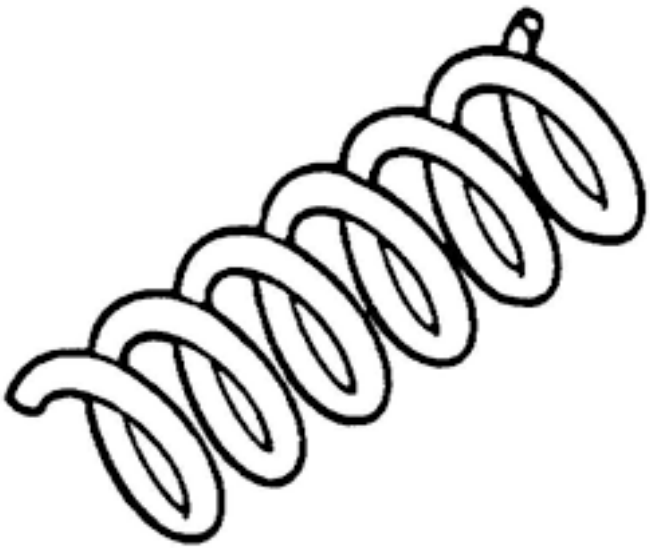
T = F/(cos 60 degrees) = F/0.5

T = F/(cos 75 degrees) = F/0.26

T = F/(cos 90 degrees) = F/0

Thus, when the pilus is oriented orthogonally to the axis of force (90 degrees), an infinite amount of tension would be required to counteract the force. This is similar to the principle that we explored in an earlier lecture (Week_02) when we looked at the amount of tension that would be required to counteract internal turgor pressure in large cells versus small cells: compared to small cells, big cells require more tension in the cell wall to counteract the same amount of internal turgor pressure.

Type IV pili behave like linear-rate (Hookean) springs



F = -*k* * d (Hooke's law)

Hooke's law of elasticity is an approximation that states that the extension of a spring is in direct proportion with the load applied to it. Many materials obey this law, as long as the load does not exceed the material's elastic limit. Materials for which Hooke's law is a useful approximation are known as linear-rate (linear-elastic) or "Hookean" materials. Hooke's law in simple terms says that strain is directly proportional to stress. Mathematically, Hooke's law states that

$F = -k * d$

where

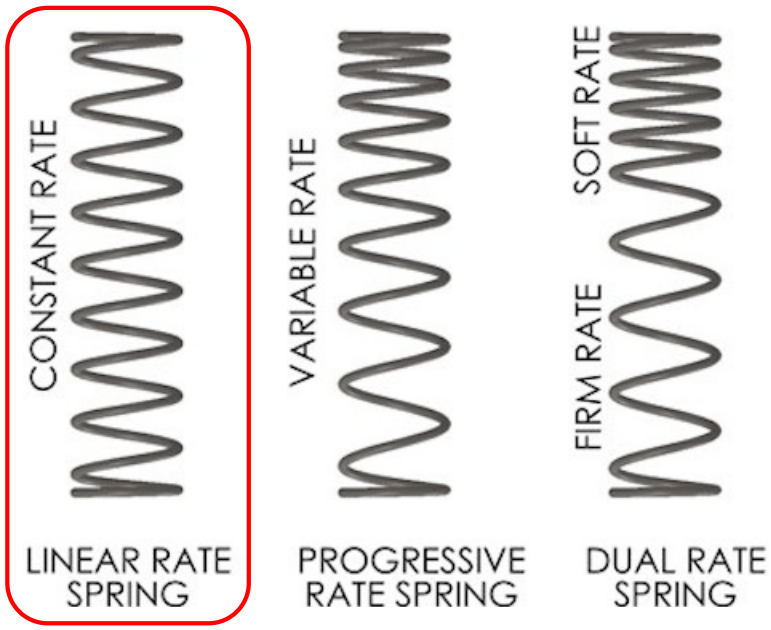
F is the restoring force exerted by the material in SI units (N or kg * m * s^{-2})

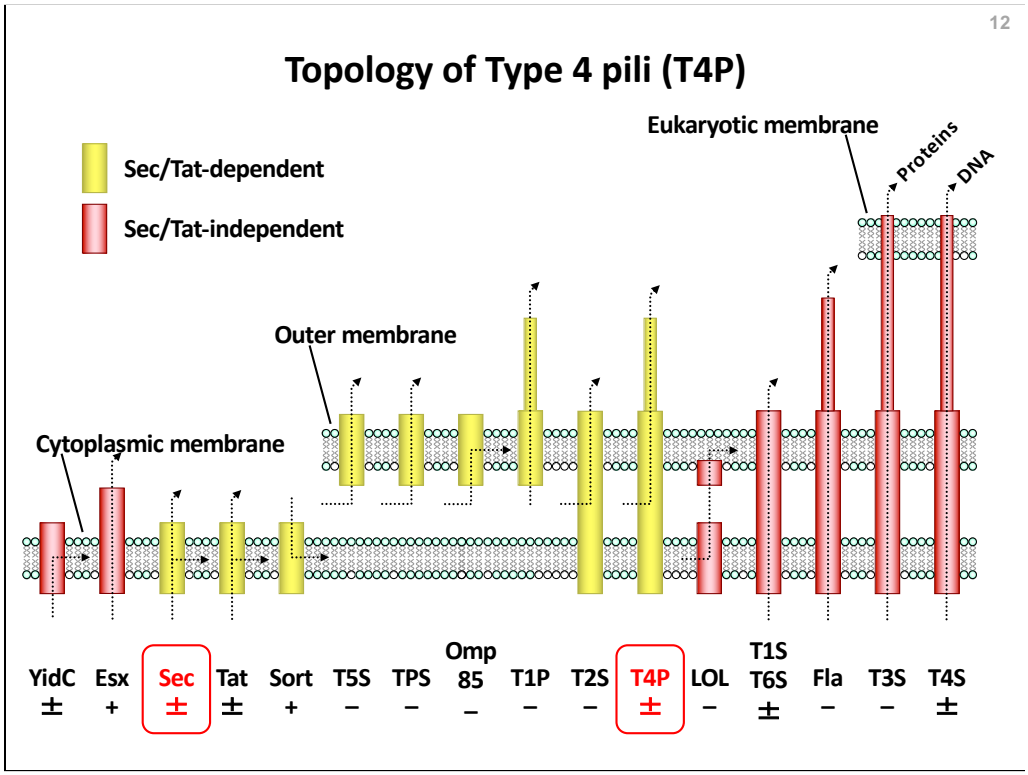
k is a constant called the "rate constant" or "spring constant" in SI units (N * m^{-1} or kg * s^{-2})

d is the displacement of the end of the spring from its equilibrium position in SI units (m)

When this holds, the behavior is said to be linear. If shown on a graph, the line should show a direct variation. There is a negative sign on the right-hand side of the equation because the restoring force always acts in the opposite direction of the displacement (for example, when a spring is stretched to the left, it pulls back to the right).

Type IV pili behave like linear-rate (Hookean) springs

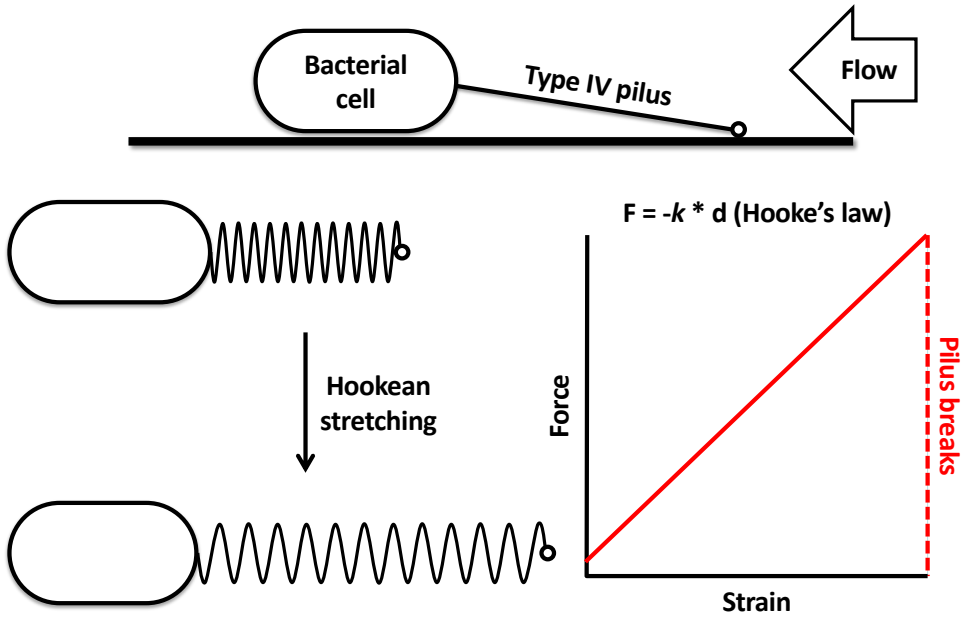


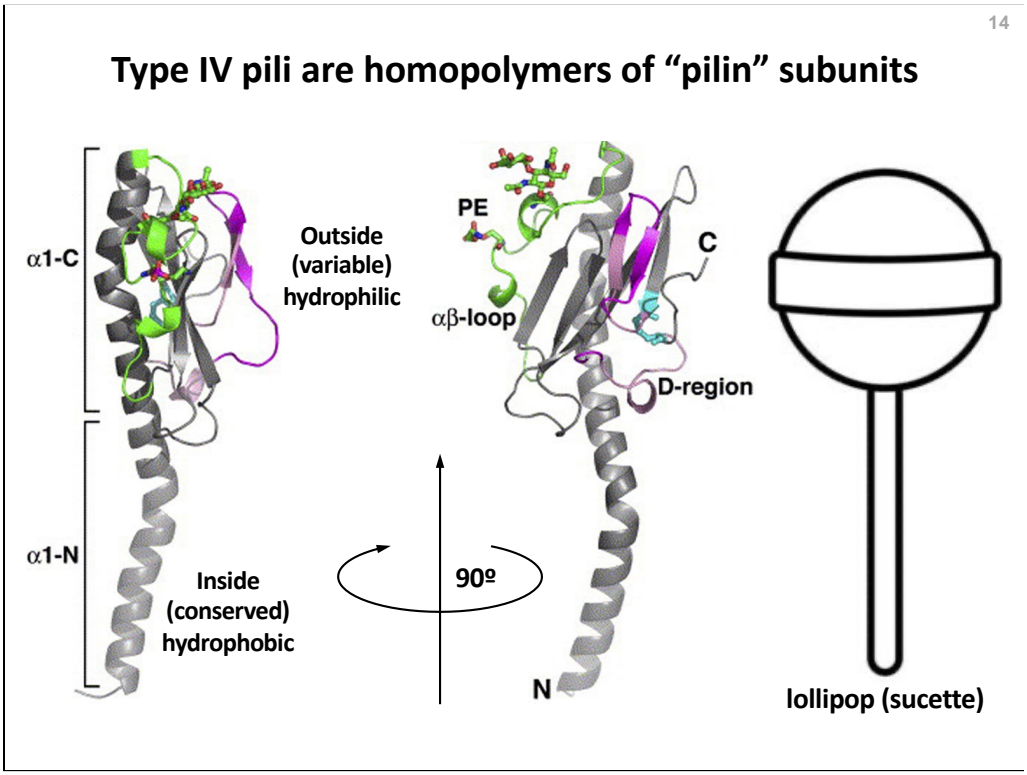


Source: Papanikou W, Karamanou S, Economou A (2007) Bacterial protein secretion through the translocase nanomachine. *Nat Rev Microbiol* 5(11): 839-851 PMID:17938627.

Note the topology of type IV pili (T4P): the base of the pilus is located in the cytoplasmic membrane, the periplasmic space, and the outer membrane, while the shaft of the pilus extends into the extracellular space. Although type IV pili extend through the cytoplasmic membrane, they nonetheless require the Sec secretion system to transfer protein subunits from the cytoplasm to the periplasmic space for their assembly.

The force-strain relationship is linear for type IV pili

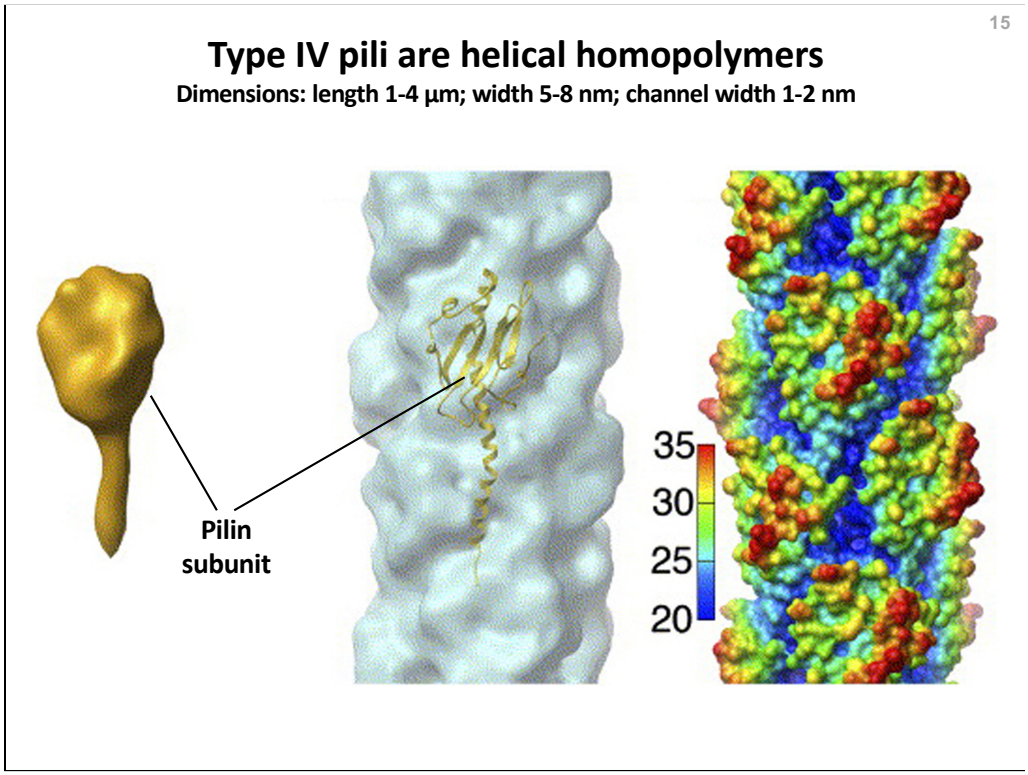




Source: Craig L, Volkmann N, Arvai AS, Pique AS, Yeager M, Egelman EH, Tainer JA (2006) Type IV pilus structure by cryo-electron microscopy and crystallography: implications for pilus assembly and functions. *Mol Cell* 23(5):651-662 PMID:16949362.

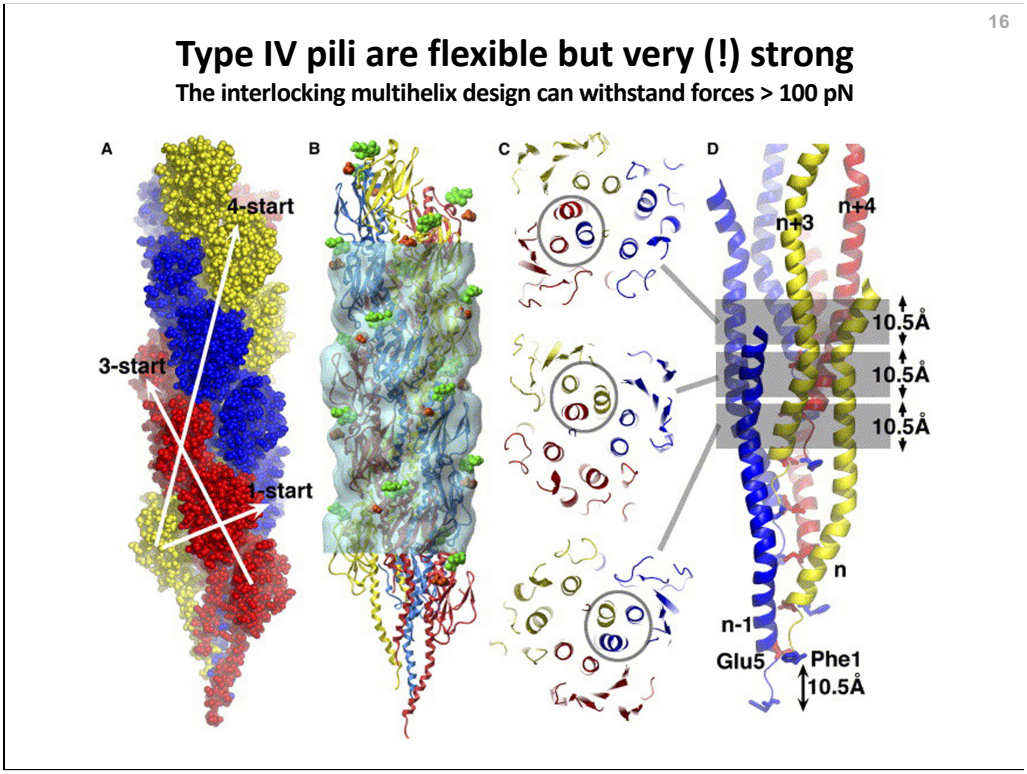
Figure 2A. Full-Length *Neisseria gonorrhoeae* pilin crystal structure. Overall, the pilin subunit has a "lollipop" ("sucette") structure with a globular "candy" part that is hydrophilic and an elongated "stick" part that is hydrophobic. The globular part is highly variable between different species and is exposed on the surface of the type IV pilus. The elongated part is highly conserved between different species and is buried within the interior of the type IV pilus, where the hydrophobic amino acid side chains are "protected" from interacting with water.

(A) Side (left) and front (right) pilin subunit views show regions conserved among the type IV pilins (gray), including the extended N-terminal helix α1 (comprising α1-N and α1-C), the β sheet, and the structurally variable D region (pink) with the hypervariable loop on the β hairpin (magenta) and the αβ loop (green) decorated with the disaccharide Gal-DADDGlc and phosphoethanolamine (PE).



Source: Craig L, Volkmann N, Arvai AS, Pique AS, Yeager M, Egelman EH, Tainer JA (2006) Type IV pilus structure by cryo-electron microscopy and crystallography: implications for pilus assembly and functions. *Mol Cell* 23(5):651-662 PMID:16949362.

Figure 3. Cryo-electron microscopy and 3D reconstruction of *Neisseria gonorrhoeae* type IV fimbriae at 12.5 Å Resolution. **(F, left)** *Neisseria gonorrhoeae* pilin subunit electron density filtered to 12.5 Å resolution. **(G, middle)** Pilin subunit match to cryo-electron microscopy density map. **(H, right)** Radial distance coloring of the resultant *Neisseria gonorrhoeae* type IV fimbriae structure showing the pronounced grooves (blue) between the globular head domains of neighboring subunits. The reconstruction contour level matches the filament model molecular surface.



Source: Craig L, Volkmann N, Arvai AS, Pique AS, Yeager M, Egelman EH, Tainer JA (2006) Type IV pilus structure by cryo-electron microscopy and crystallography: implications for pilus assembly and functions. *Mol Cell* 23(5):651-662 PMID: 16949362.

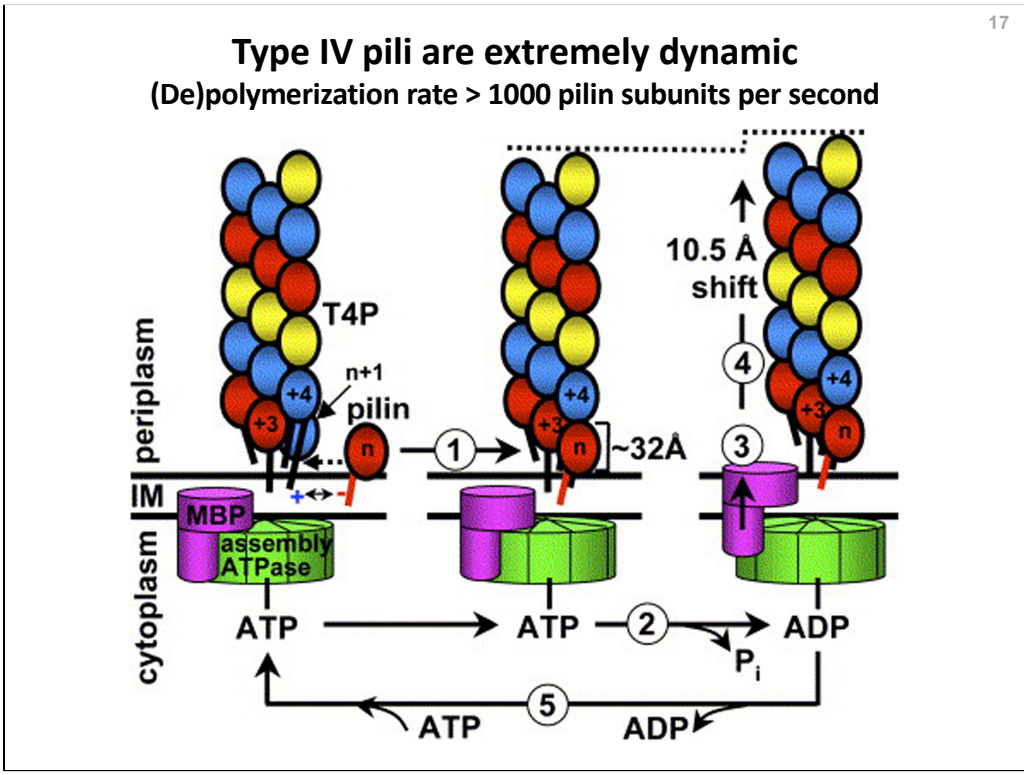
Source: Craig L, Pique ME, Tainer JA (2004) Type IV pilus structure and bacterial pathogenicity. *Nature Rev Microbiol* 2(5): 363-378 PMID: 15100690.

Figure 4. High-resolution model of the *Neisseria* type IV pilus filament. **(A)** Subunits traced along a right-handed 1-start helix, along three strands of a left-handed 3-start helix (colored red, blue, and yellow), and along four strands of a right-handed 4-start helix. **(B)** Pilin subunits of GC-T4P within the isosurface of the cryo-EM density map. **(C)** Pilin subunit packing of alternating 3-helix bundles that wind up the filament axis and exchange partners every 10.5 Å, matching the subunit axial rise for the 1-start helix. Three consecutive 10.5 Å slabs of the filament illustrate the alternating nature of the 3-helix bundles (circled). **(D)** N-terminal α helices (side view) showing the pairing every 10.5 Å of negatively charged Glu5 of one subunit (n-1 in this case) and the positively charged N-terminal Phe1 of the next subunit up (n) in the 1-start helix.

Question: How strong are Type IV pili? They have been shown to withstand tensile forces of at least 100 pN (10^{-10} N). For simplicity, we consider the Type IV pilus to have a square cross-section that is 10 nm on a side, which means the area of the cross-section is (10 nm) * (10 nm) = 100 nm^2. (A Type IV pilus actually has a circular cross-section 5-8 nm in diameter, but this estimate is good enough for our purposes and using it will actually *underestimate* the true breaking strength of the pilus.) Thus, the Type IV pilus can withstand tensile stress of at least: (100 pN) * $(100\ nm^2)^{-1}$ = $(10^{-10}$ N) * $(10^{-16}\ mm^2)^{-1}$ = 10^6 N * mm^{-2} = **1,000,000 MPa** (remember, 1 N * mm^{-2} = 1 N * 10^{-6} m^2 = 1 MPa).

For comparison, a cable made from high-quality steel has a breaking strength of about **2,000 MPa**. I obtained this value from the "Wire Rope Capacity Charts" available online at http://www.cglift.com/product-catalog/wire-rope/.

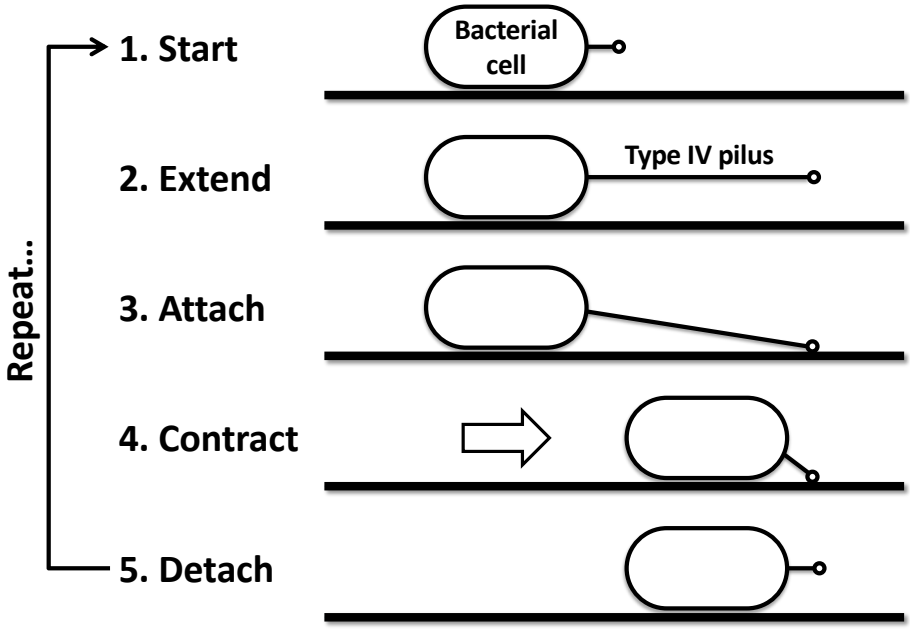
Answer: Type I pili are very, very strong!



Source: Craig L, Volkmann N, Arvai AS, Pique AS, Yeager M, Egelman EH, Tainer JA (2006) Type IV pilus structure by cryo-electron microscopy and crystallography: implications for pilus assembly and functions. *Mol Cell* 23(5):651-662 PMID: 16949362.

Figure 6. Proposed type IV pilus assembly mechanism. At the cytoplasmic membrane a.k.a. inner membrane (IM), pilin subunits are solubilized in the membrane by complementarity of their hydrophobic N-terminal alpha-helix with the lipid bilayer and positively charged globular domain bottoms with the negatively charged phospholipid head groups. Subunits add to the growing filament, in part by charge complementarity between Glu5 of the incoming subunit (n) and Phe1 of the existing n+1 subunit, numbered along the 1-start helix. **(1)** Subunit n binds to the growing filament. **(2)** One molecule of ATP, which is bound to the hexameric assembly ATPase on the cytoplasmic side of the IM, is hydrolyzed. **(3)** ATP hydrolysis induces a piston-like motion in the ATPase membrane binding partner (MBP). **(4)** This motion drives the growing filament outward toward the periplasm by a distance of 10.5 Å. **(5)** ADP is exchanged for ATP, allowing the assembly apparatus to relax to its resting position. This mechanism may occur consecutively at three active sites on the ATPase, one for each strand of the 3-start helix (shown here in red, blue, and yellow). Three consecutive ATP hydrolysis events would push the filament out 10.5 Å, resulting in a 31.5 Å gap at the first site to accommodate a new subunit.

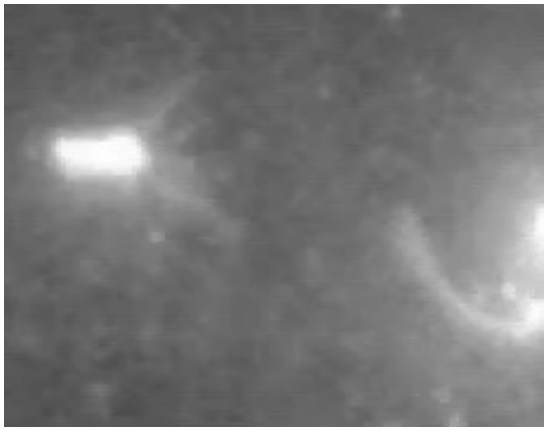
Type IV pili drive "twitching motility" on surfaces



"Twitching motility": movement of bacteria on surfaces by extension, attachment, and retraction of type IV pili

Pilus not attached to surface (pili retract)

Pilus attached to surface (cell moves)

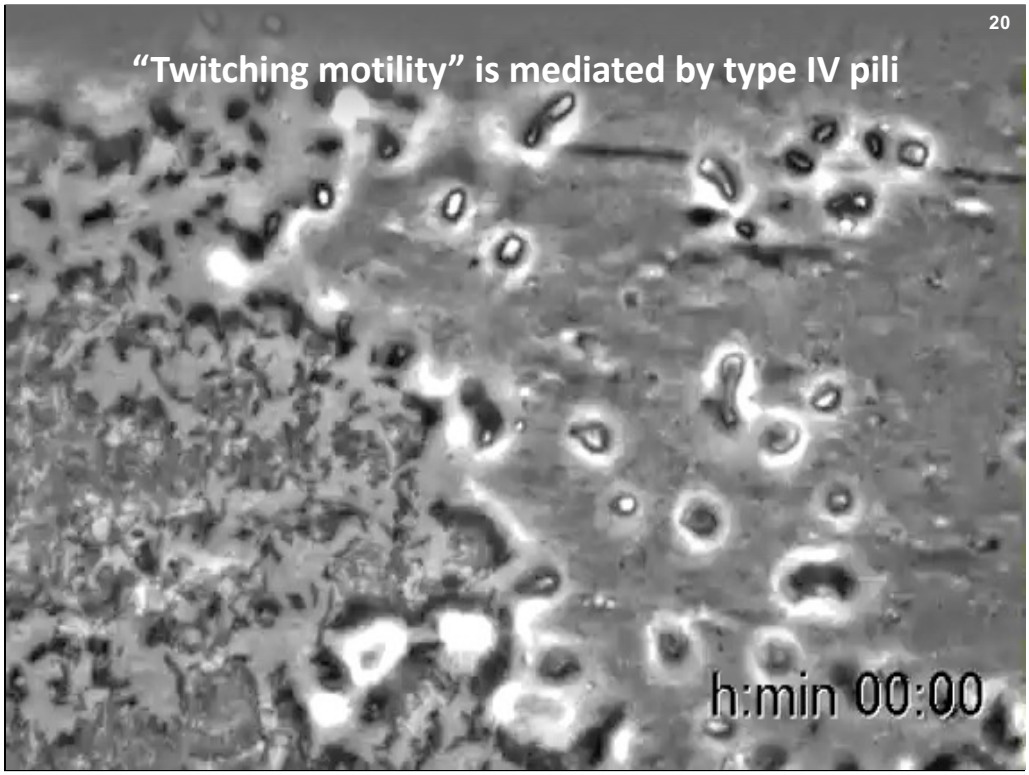


See: Movie_Slide19a.mov posted on Moodle.

See: Movie_Slide19b.mov posted on Moodle.

Source: http://www.rowland.harvard.edu/labs/bacteria/movies_paeru.html

Twitching motility in *Pseudomonis* aeruginosa. Type IV pili are thin (6 nm dia.) filaments that are essential for twitching motility in many bacteria. How do Type IV pili generate cell movement? The first clues came from electron microscopy studies of bacteriophage infection of a variety of Gram-negative bacteria. After the initial interaction between the phage and the pilus, it appeared that retraction pulled the phage to the cell surface where productive infection could occur. Using optical tweezers, Mertz *et al.* (2000) demonstrated that Type IV pili could retract with considerable force (> 80 pN). Mutants that were pili-less or had non-retractile pili were non-motile; thus pilus retraction powers twitching motility. The pili were not observed directly - pilus function was assayed by monitoring the position of a latex bead attached to pili with antibodies. We developed a technique to label pili using an amino-reactive Cy3 and observed directly pilus extension, pilus retraction, and retraction-mediated cell movement (Skerker & Berg, 2000). Labeled cells were videotaped in fluorescence using evanescent wave excitation at a quartz-water interface. Pili tended to stick to the quartz at their distal tips. If the cell body also stuck, pili under retraction were pulled taut (Movie 1). If the cell body was free, retraction pulled it forward (Movie 2). The QuickTime movies shown are 10 times actual speed.

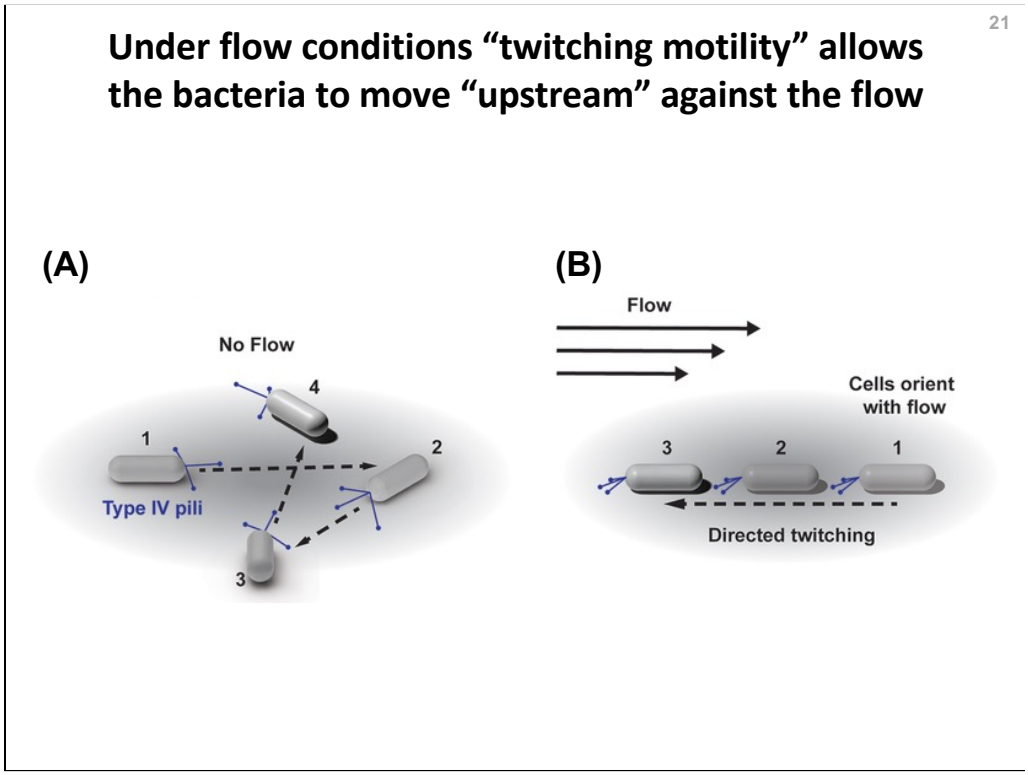


See: Movie_Slide20.mov posted on Moodle.

Source: http://www.nysaes.cornell.edu/pp/faculty/hoch/movies/

Twitching motility in *Xylella fastidiosa*. Individual cells, grown on cellophane overlaid on nutrient agar, are shown moving within the confines of a colony margin via “twitching motility” mediated by Type IV pili.

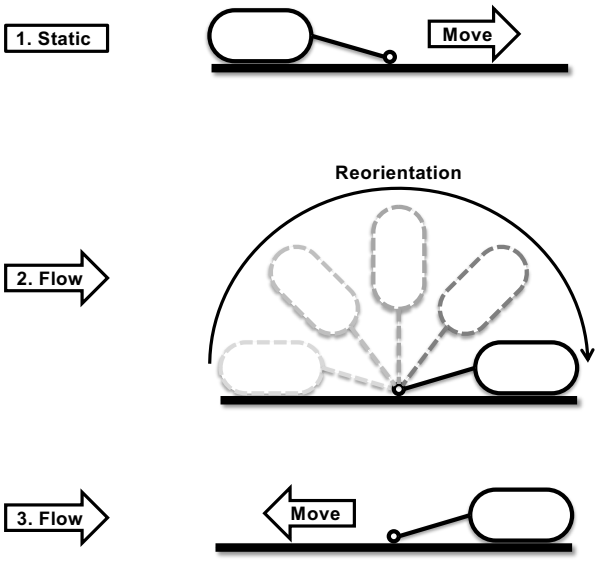
https://www.youtube.com/watch?v=m1vJKz_bV7U



Source: Padron GC, Shuppara AM, Palalay JS, Sharma A, Sanfilippo JE (2023) Bacteria in fluid flow. *J Bacteriol* 205(4): e0040022 PMID: 36951552.

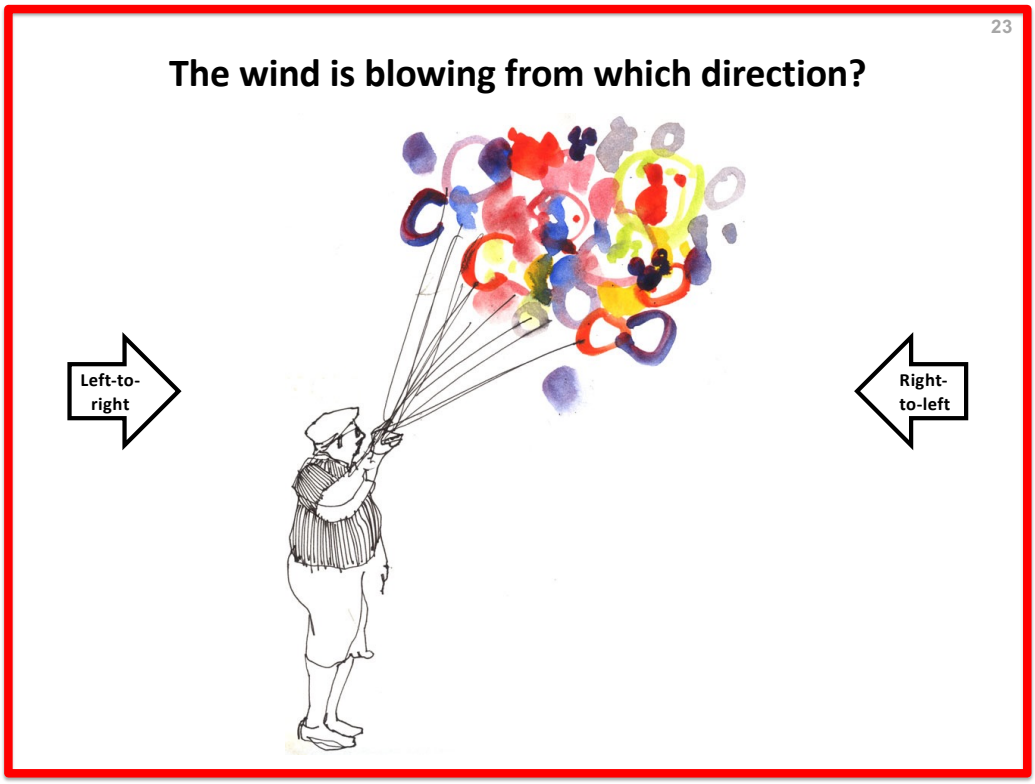
Figure 3. Bacterial cells use extension and retraction of type IV pili (shown in blue) to move on surfaces via “twitching motility”. **(A)** In conditions without flow, twitching motility typically does not proceed in a directed manner. **(B)** In conditions with flow, cells are reoriented along the axis of flow, which leads to directed movement against the direction of flow.

Reorientation of T4P-tethered bacteria under flow



Shen Y, Siryaporn A, Lecuyer S, Gitai Z, Stone HA (2012) Flow directs surface-attached bacteria to twitch upstream. *Biophys J* 103(1): 146-151 PMID: 22828341.

Abbreviation: T4P, type IV pili.



Answer: From left to right.

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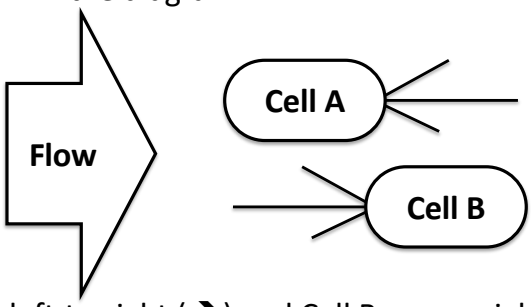
Type IV pili are clustered at one cell pole. In the absence of flow, Cell A moves left-to-right and Cell B moves right-to-left. If flow is applied as shown in the diagram:



A. Cell A moves left-to-right (➔) and Cell B moves right-to-left (🡐).

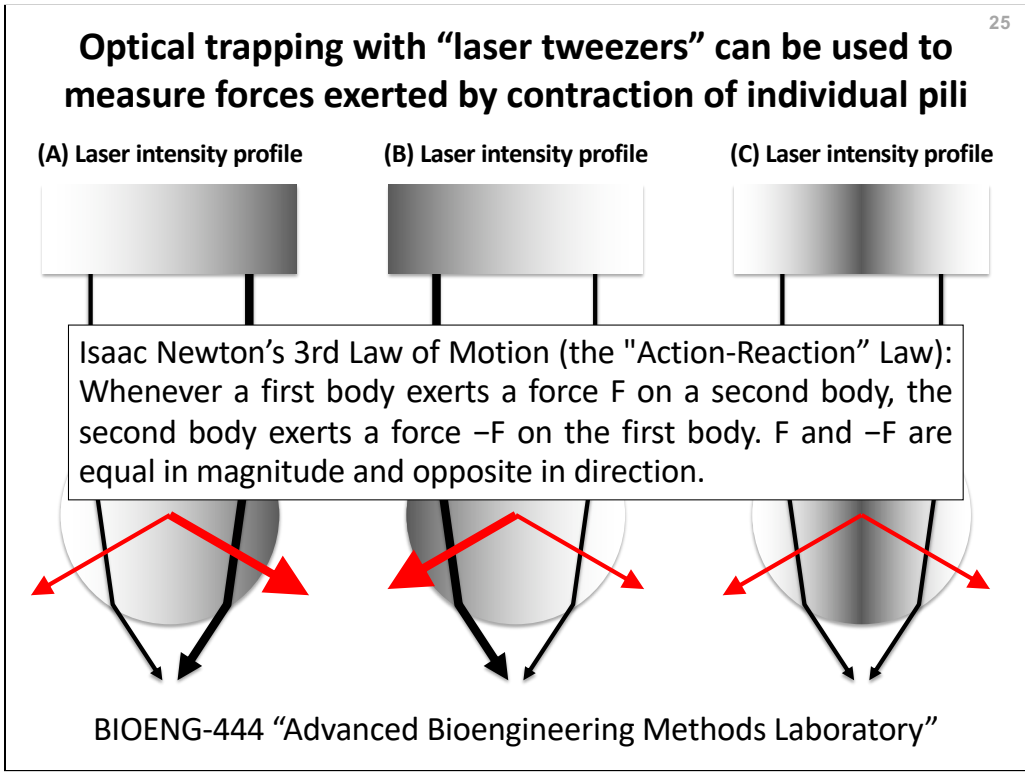
B. Cell A moves right-to-left (🡐) and Cell B moves left-to-right (➔).

C. Cell A and Cell B both move left-to-right (➔).

D. Cell A and Cell B both move right-to-left (🡐).

Answer: (D)

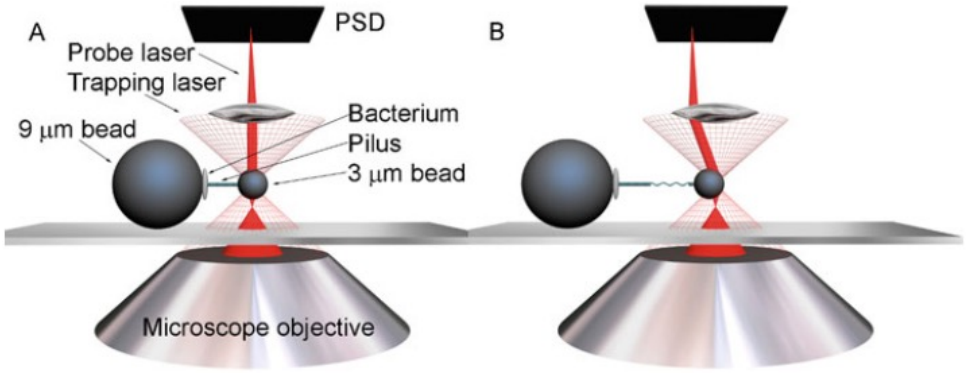
This answer may seem counter-intuitive! But remember: Type IV pili are localized to just one pole of the bacterial cell, as depicted in the drawing. This point is critical. Imagine that the bacterial cell is like a “balloon” floating above the surface. Now imagine that the bacterial cell is attached to the surface *at one cell pole* by a few type IV pili acting like “strings”. In sum: the strings are attached to the surface and the balloon is floating above the surface. Now, if you apply flow from left to right, the body of the balloon will be pushed downstream by the flow, thus orienting the balloon with the attached strings pointing *upstream*. Now, with this orientation, if the strings extend, attach, and retract (which is what type IV pili do), the balloon will “twitch” across the surface in the *upstream* direction! Which is exactly what many pathogenic bacteria “want” to do: to crawl against the flow of body fluids that is meant to remove bacteria (and other nasty things, like viruses, dirt, wastes, etc.) from the affected organ.



Source: Neuman KC, Block SM (2004) Optical trapping. *Rev Sci Instrum* 75(9): 2787-2809 PMID: 16878180.

Figure 1. Ray optics description of light gradient force. (A-B) A transparent bead is illuminated by a parallel beam of light with an intensity gradient that increases (A) or decreases (B) from left to right. Two representative rays of light of different intensities (represented by black lines of different thickness) from the beam are shown. The refraction of the rays by the bead changes the momentum of the photons, equal to the change in the direction of the input and output rays. Conservation of momentum dictates that the momentum of the bead changes by an equal but opposite amount, which results in the forces depicted by gray arrows. The net force on the bead is to the right (A) or left (B), in the direction of the intensity gradient, and slightly down. (C) To form a stable trap, the light must be focused, producing a three-dimensional intensity gradient. In this case, the bead is illuminated by a focused beam of light with a radial intensity gradient. Two representative rays are again refracted by the bead but the change in momentum in this instance leads to a net force towards the focus. Gray arrows represent the forces. The lateral forces balance each other out and the axial force is balanced by the scattering force (not shown), which decreases away from the focus. If the bead moves in the focused beam, the imbalance of optical forces will draw it back to the equilibrium position.

Type IV pili generate strong nanoscale forces (>100 pN)
(this is about 20-fold higher than kinesin or myosin motors)

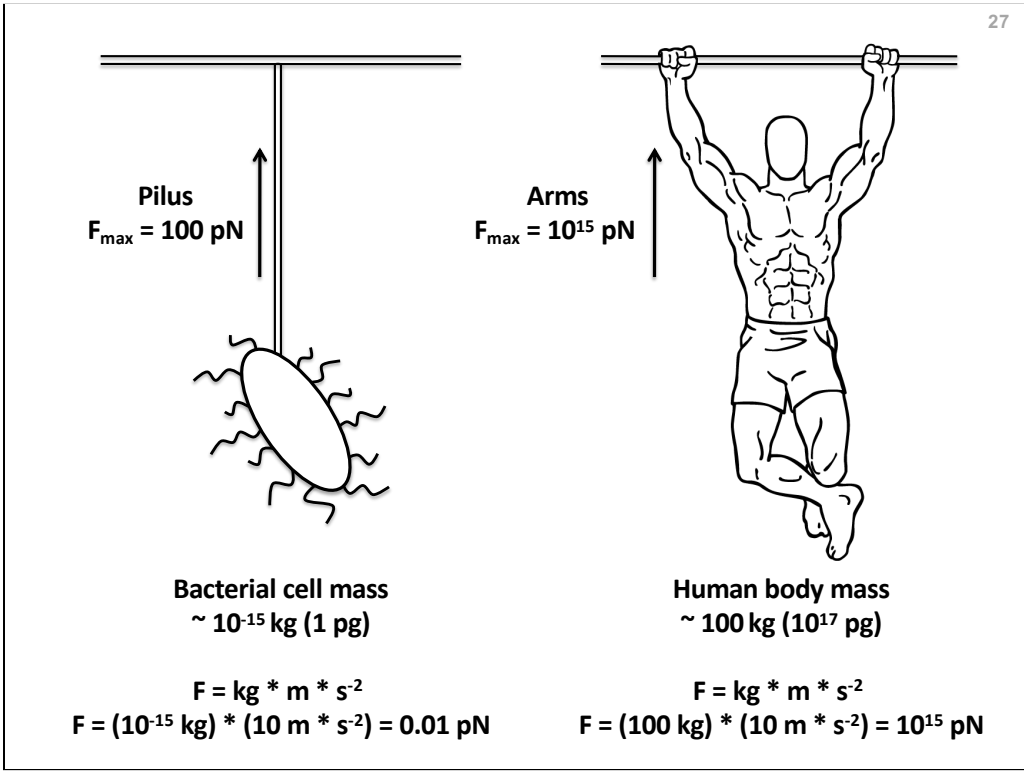


BIOENG-444 "Advanced Bioengineering Methods Laboratory"

Source: Axner A, Andersson M, Björnham O, Castelain M, Klinth J, Koutris E, Schedin S (2011) Assessing bacterial adhesion on an individual adhesin and single pili level using optical tweezers. *Adv Exp Med Biol* 715: 301-313 PMID:21557072.

Figure 19.1. Schematic of FMOT (force-measuring optical tweezers) for assessment of the biomechanical properties of pili. (**A**) A single bacterium is mounted on a 9 μm large bead that is firmly attached to the microscope slide. A free-floating single bacterium is trapped by the optical tweezers at low laser power and mounted onto the bead. A trapped bead, whose position is probed by a separate probe laser, and to which one or several pili are attached, is used as a force transducer. (**B**) Strain is applied by slowly translating the microscope slide. The force is assessed by detecting the deflection of the probe light by a position sensitive detector (PSD) whose output is converted to a force by a calibration process. Dimensions not to scale.

Force (F) = N = kg * m * s^{-2}.



A bacterial cell weighs about 1 pg. A type IV pilus can exert a force of about 100 pN. This means that a bacterial cell could "lift" 10,000-times its own body mass against gravity using a single pilus. The equivalent for a human would be (100 kg) * (10,000) = 1,000,000 kg. Imagine doing a one-arm pullup with a 1,000,000 kg weight attached to your ankles...that would be impressive! ;-)

For these “back of the envelope” calculations, I used an acceleration of 10 m * s^{-2}, which is the approximate value for the rate of acceleration due to one Earth gravity (close enough for biology...).

Force is expressed in SI units of (kg * m * s^{-2}).

The force required to “pullup” a bacterial cell weighing 1 pg (10^{-15} kg) against the force of gravity is:

F = mass * acceleration = (10^{-15} kg) * (10 m * s^{-2}) = 10^{-14} kg * m * s^{-2} = 10^{-14} N = 0.01 pN

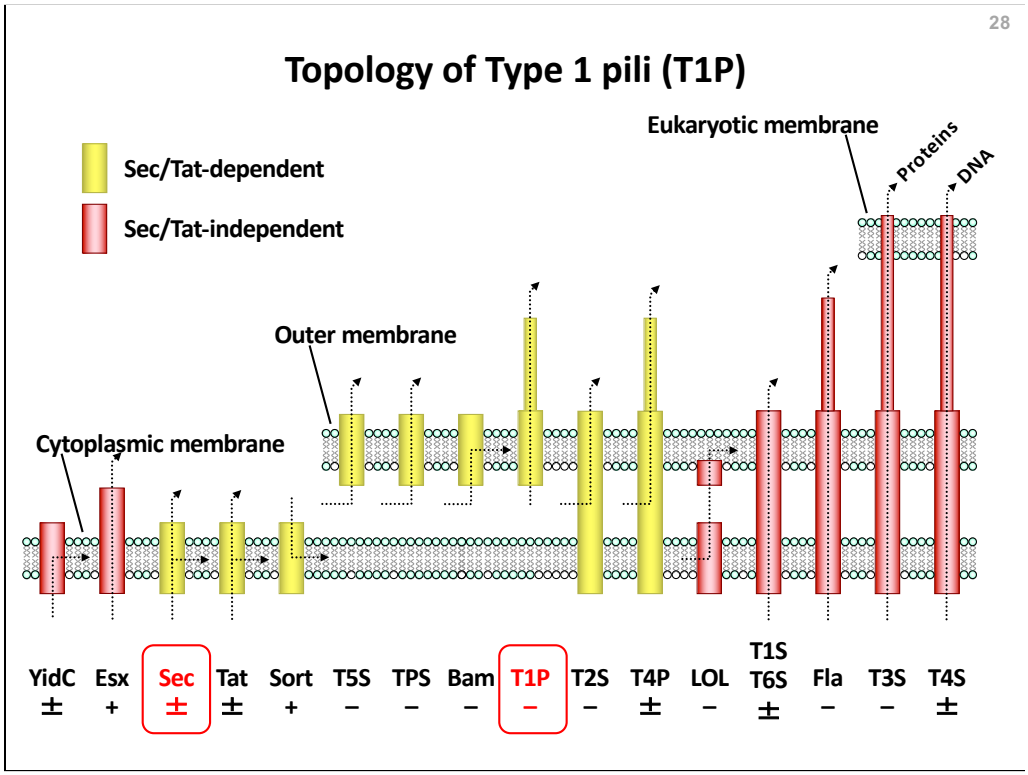
A type IV pilus can exert a maximum force (F_{max}) of about 100 pN. This is enough force to “pullup” 10,000 bacterial cells (10,000 pg) against the force of gravity.

The force required to “pullup” a human body weighing 100 kg (10^{17} pg) against the force of gravity is:

F = mass * acceleration = (100 kg) * (10 m * s^{-2}) = 10^{3} kg * m * s^{-2} = 10^{3} N = 10^{15} pN.

If a human doing a one-arm pullup could lift the equivalent of 10,000 bodies against the force of gravity, this would be equivalent to (100 kg) * (10,000) = 1,000,000 kg!

In sum: bacteria are small but mighty, and pili are awesomely strong! Relative to their size, of course...

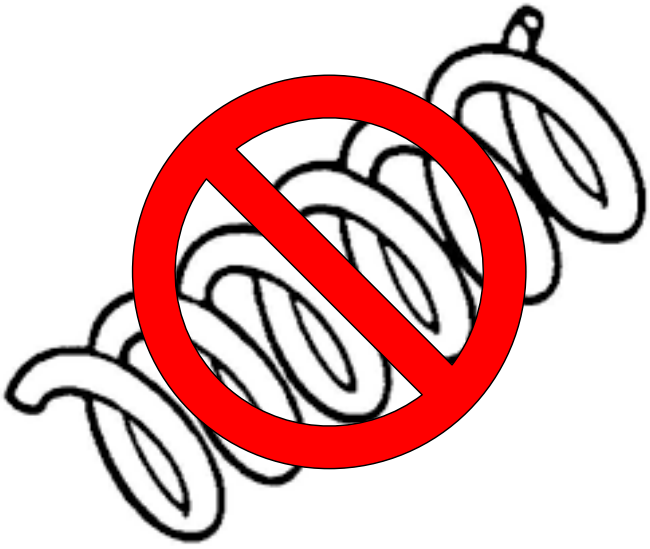


Source: Papanikou W, Karamanou S, Economou A (2007) Bacterial protein secretion through the translocase nanomachine. *Nat Rev Microbiol* 5(11): 839-851 PMID:17938627.

Note the topology of type I pili (T1P): the base of the pilus is located in the outer membrane and the shaft of the pilus extends into the extracellular space. Type I pili do not extend through the cytoplasmic membrane. Therefore, assembly of Type I pili is dependent on transfer of protein subunits from the cytoplasm to the periplasmic space mediate by the Sec secretion system (Sec), which is located in the inner (cytoplasmic) membrane.

29

Type I pili behave like dual-rate (non-Hookean) springs



F = -*k* * d (Hooke's law) does not apply!

Hooke's law of elasticity is an approximation that states that the extension of a spring is in direct proportion with the load applied to it. Many materials obey this law, as long as the load does not exceed the material's elastic limit. Materials for which Hooke's law is a useful approximation are known as linear-rate (linear-elastic) or "Hookean" materials. Hooke's law in simple terms says that strain is directly proportional to stress. Mathematically, Hooke's law states that

$F = -k * d$

where

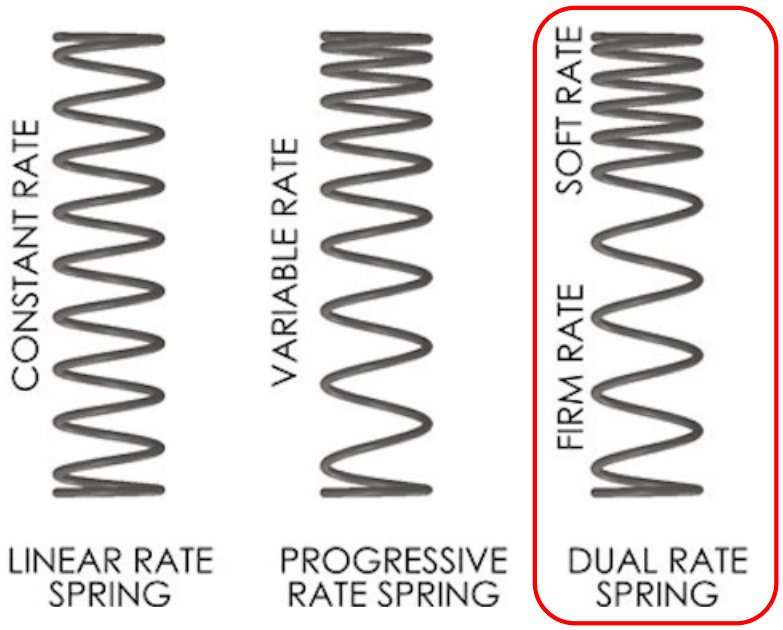
F is the restoring force exerted by the material in SI units (N or kg * m * s^{-2})

k is a constant called the rate constant or spring constant in SI units (N * m^{-1} or kg * s^{-2})

d is the displacement of the end of the spring from its equilibrium position in SI units (m)

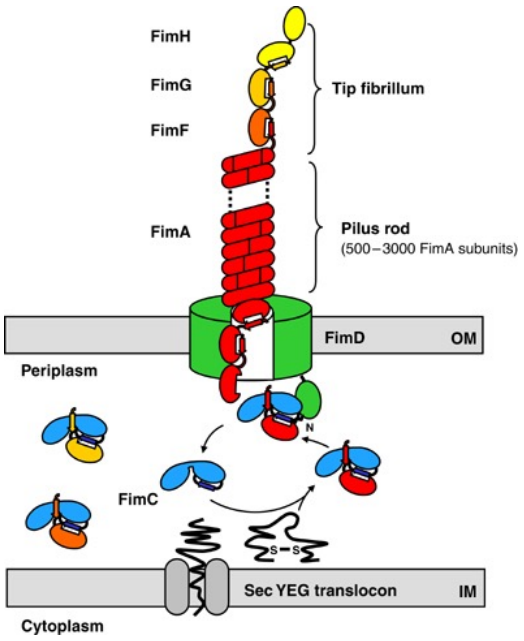
When this holds, the behavior is said to be linear. If shown on a graph, the line should show a direct variation. There is a negative sign on the right-hand side of the equation because the restoring force always acts in the opposite direction of the displacement (for example, when a spring is stretched to the left, it pulls back to the right).

Type I pili behave like dual-rate (non-Hookean) springs



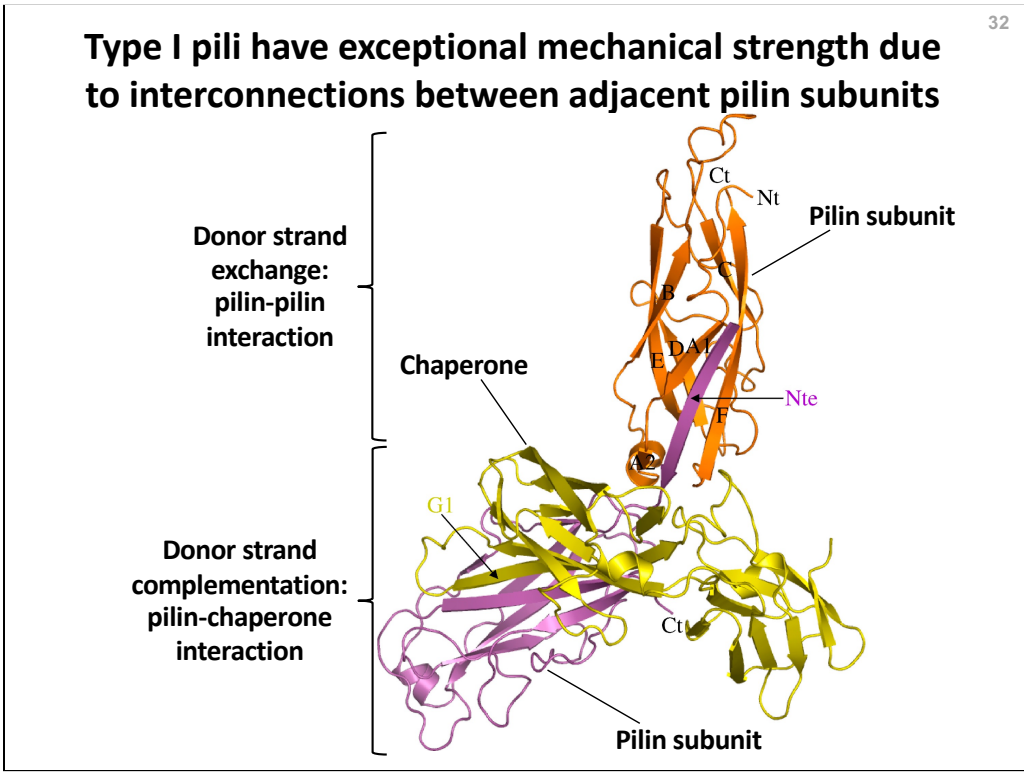
Type I pili are self-secreting and self-assembling

- FimH - tip adhesin
- FimG - tip pilin
- FimF - tip pilin
- FimA - rod pilin
- FimD - translocase
- FimC - chaperone



Source: http://2008.igem.org/Team:NYMU-Taipei/Project/Attachment

Type I pili of uropathogenic *Escherichia coli* (UPEC) strains provide a paradigm for self-assembly, structure, and function of bacterial adhesive pili. UPEC are the most important cause of acute cystitis (bladder infections) and pyelonephritis (kidney infections) and the number one reason for prescription of antibiotics worldwide. Type I pili are important for colonization of the bladder because they bind with high affinity and specificity to mannose residues decorating the protein Uroplakin 1a. This protein is highly abundant on the surface of Umbrella Cells, which comprise the most superficial layer of cells in the bladder epithelium surrounding the bladder lumen. Binding of UPEC to the bladder epithelium prevents them from being flushed away during micturition (a.k.a. "peeing") and promotes the invasion of the bladder epithelium by UPEC. Indeed, the binding strength of Type I pili to the ligand (mannose residues on Uroplakin 1a) is actually *increased* by low-to-moderate shear stress, a phenomenon known as *catch-bond adhesion*. By contrast, the strength of "typical" receptor-ligand interactions is *decreased* by shear stress at any level (low, moderate, or high), a phenomenon known as *slip-bond adhesion*. Catch-bond adhesion is a very cool engineering trick! We'll have more to say about it later in this lecture.



Source: Verger D, Bullitt E, Hultgren SJ, Waksman G (2007) Crystal structure of the P pilus rod subunit PapA. *PLoS Pathog* 3(5):e73 PMID: 17511517.

Source: Aprikian P, Interlandi G, Kidd BA, Le Trong I, Tchesnokova V, Yakovenko O, Whitfield MJ, Bullitt E, Stenkamp RE, Thomas WE, Sokurenko EV (2011) The bacterial fimbrial tip acts as a mechanical force sensor. *PLoS Biol* 9(5): e1000617 PMID: 21572990.

Figure 2. Three-dimensional crystal structure of the pilin-chaperone assembly complex shown in ribbon representations with beta-strands indicated as arrows and alpha-helices indicated as cylinders: chaperone (yellow), pilin subunit in the donor-strand-complemented form (purple), and pilin subunit in the donor-strand-exchange form (orange). Pilin subunits are basically a beta-barrel with one "missing" beta-strand. In **donor strand complementation**, a beta-strand from the chaperone (G1 in yellow) inserts into the "incomplete" beta-barrel of the docked pilin subunit, thereby generating a "complete" beta-barrel. In **donor strand exchange**, a beta-strand from one pilin (Nte in purple) inserts into the "incomplete" beta-barrel of the adjacent pilin subunit, thereby generating a "complete" beta-barrel. Thus, the fully assembled Type I pilus is like a chain of lollipops, where each pilin subunit is shaped like a lollipop and the "stick" of one lollipop inserts into the "disk" of the next lollipop in the chain. These interpenetrating interactions between adjacent pilin subunits confer exceptional resistance to tensile stresses acting along the long axis of the pilus, which is ideal for an appendage that mediates attachment to surfaces. This is an exceptionally beautiful molecular superstructure!

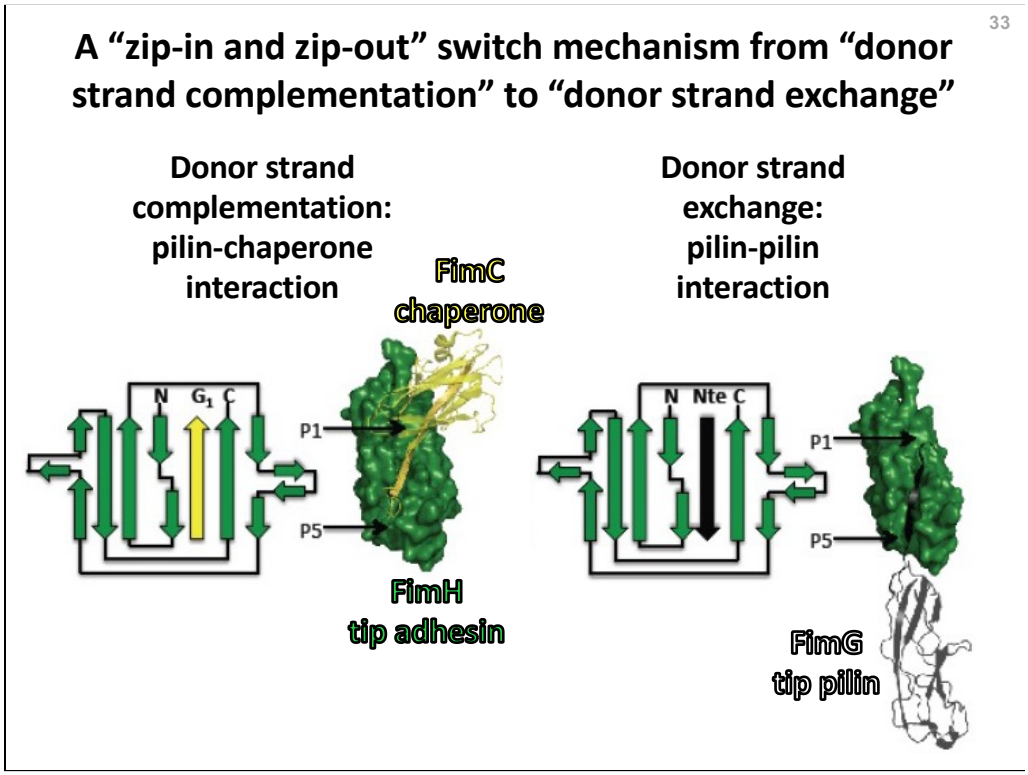
Tensile **strength** is the amount of force that a material can withstand before rupturing, measured in units of newtons per square meter (N * m^{-2}) or pascals.

Tensile **toughness** is the amount of energy per unit volume that a material can absorb before rupturing, measured in units of joules per cubic meter (J * m^{-3}).

Toughness of a Type I pilus = 10 MJ * m^{-3}

Toughness of high-tensile steel = 6 MJ * m^{-3}

Abbreviations: Ct, C-terminus; Nt, N-terminus, Nte, N-terminal extension.

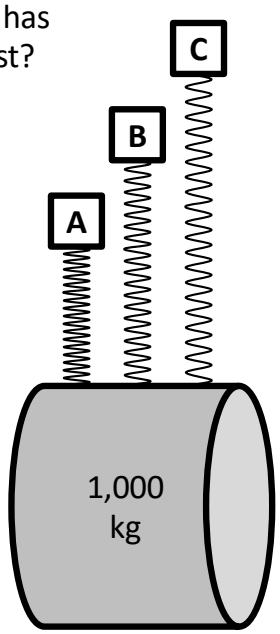


Source: Lillington J, Waksman G (2013) Ordered and ushered: the assembly and translocation of the adhesive Type I and P pili. *Biology (Basel)* 2(3): 841-860 PMID: 24833049.

Figure 1. Topology diagrams and cartoons showing ***donor-strand complementation*** in the pilin-chaperone interaction between the FimH tip adhesin subunit and the FimC chaperone (left), and ***donor-strand exchange*** in the pilin-pilin interaction between the FimH tip adhesin subunit and the FimG tip pilin subunit (right). The examples given here are FimC:FimH (yellow:green) and FimH:FimG (green:black), respectively. During the “zip-in and zip-out” transition, the beta-strand donated by the chaperone (yellow arrow, left panel) to complete the FimH beta-barrel is replaced by the beta-strand donated by the chaperone (black arrow, right panel).

34

Hooke's law states that F = -k * D. Which spring has the highest tension? Which spring will break first?



A. Spring (A).

B. Spring (B).

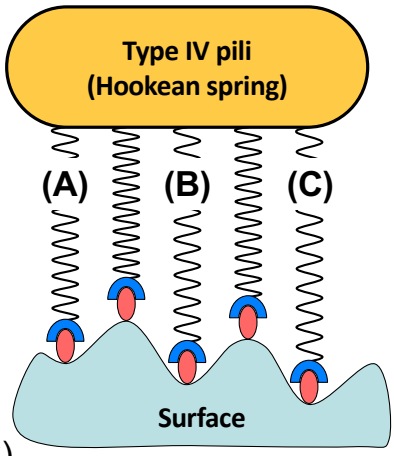
C. Spring (C).

D. Equal probability that any of the three springs will break first.

Answer: (C)

35

The five pili (A-E) are equal in length but they are stretched to different degrees. The tension is highest in:



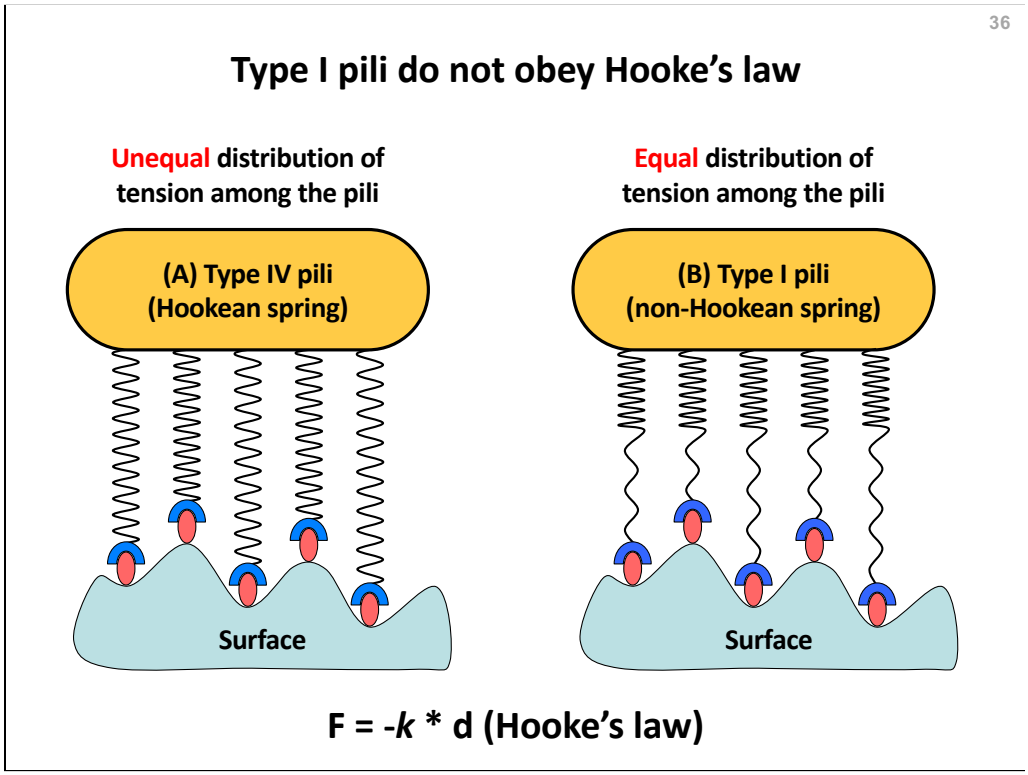
A. Pilus (A)

B. Pilus (B)

C. Pilus (C)

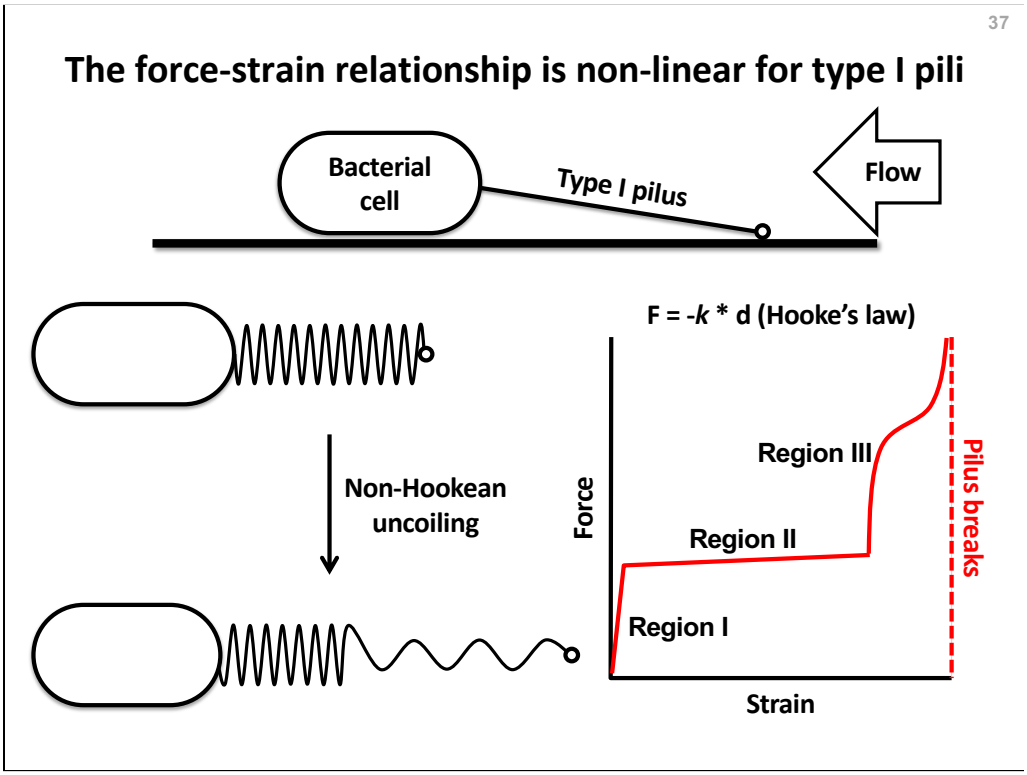
D. Tension is the same in all pili (A-C)

Answer: (C)



(A) Type IV pili behave like linear-rate Hookean springs. Under tensile stress, they extend by **stretching**.

(B) Type I pili behave like dual-rate non-Hookean springs. Under tensile stress, they extend by **uncoiling**.



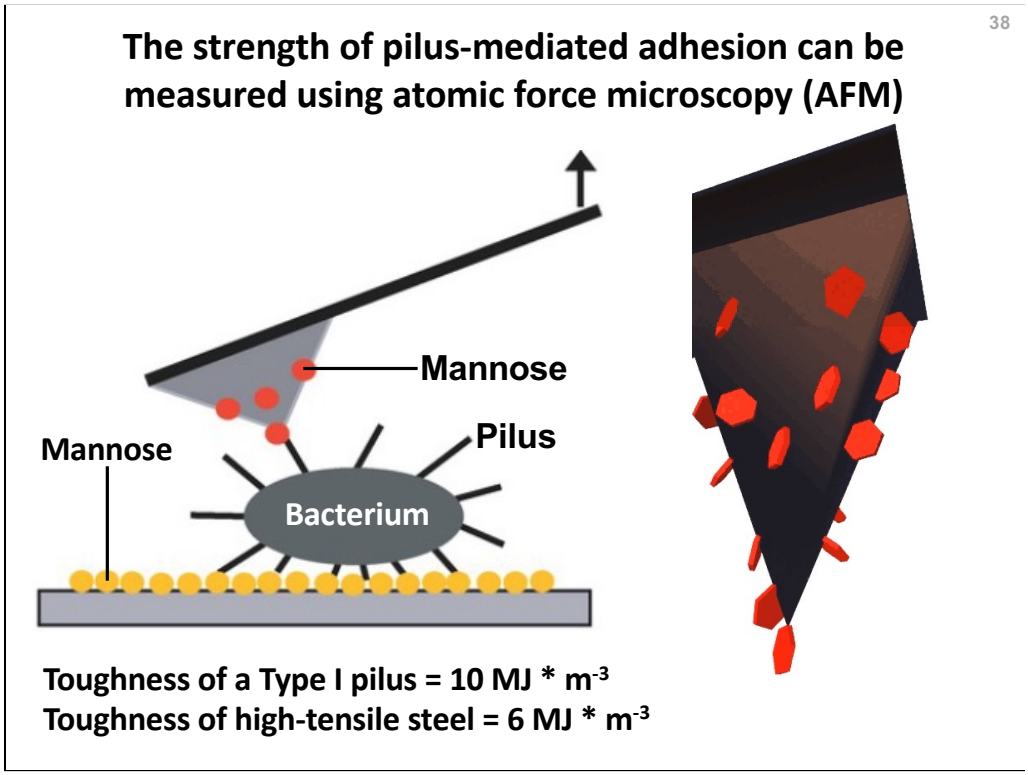
Source: Aberg V, Fällman E, Axner O, Uhlin BE, Hultgren SJ, Almqvist F (2007) Pilicides regulate pili expression in *E. coli* without affecting the functional properties of the pilus rod. *Mol Biosyst* 3(3): 214-218 PMID: 17308668.

Region I of the force curve originates from elastic stretching of the helical structure of the pilus.

Region II is due to a sequential unfolding of the layer-to-layer bonds (and thereby of the helical structure) of the pilus.

Region III, with its characteristic wave-shape, is due to an elastic stretching of the linearized pilin protein sequence, which is opposed by the head-to-tail bonds.

Does this non-linear behavior remind you of something we explored in an earlier class? Like intermediate filaments, maybe?



See: Movie_Slide38.mov posted on Moodle.

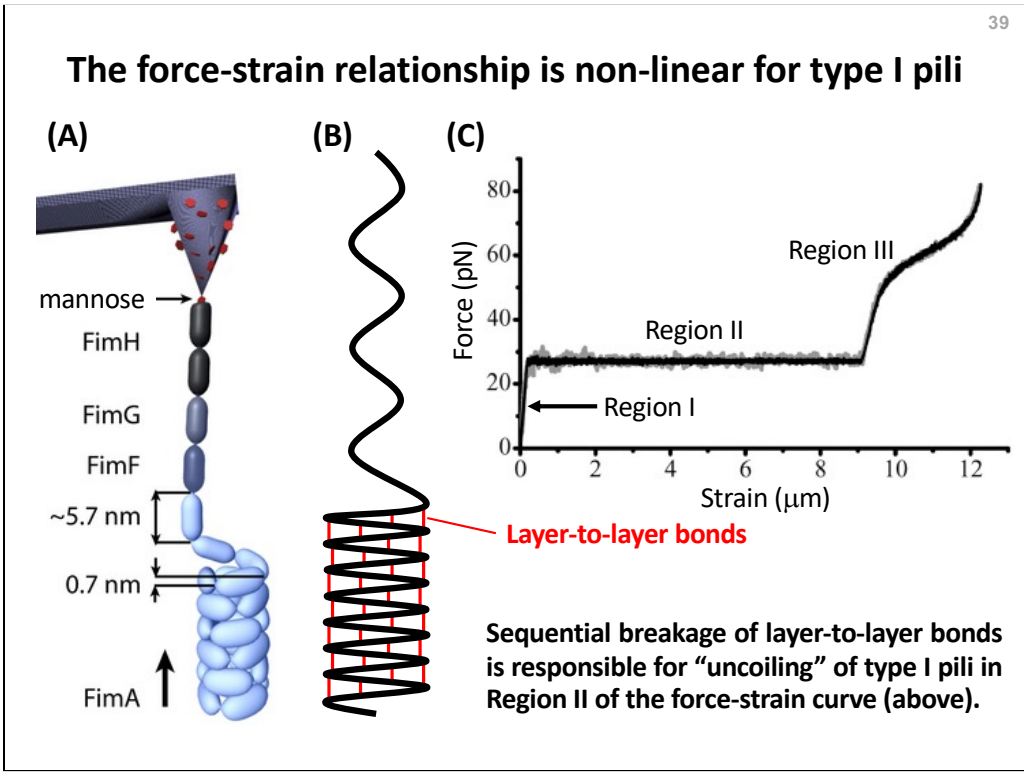
Source: Forero M, Yakovenko O, Sokurenko EV, Thomas WE, Vogel V (2006) Uncoiling mechanics of *Escherichia coli* type I fimbriae are optimized for catch bonds. *PLoS Biol.* 4(9): e298 PMID: 16933977.

Figure 1. Force measurements on Type I pili of *Escherichia coli*. **(A, left)** Experimental setup. The tip (gray) of an AFM cantilever is coated with 1-Mannose-BSA (red) or 3-Mannose-RNAseB (yellow). An *E. coli* bacterium is attached to a 3-Mannose-RNAseB-covered glass surface. After the bacterium is approached with the AFM tip, one or multiple pili can bind. The cantilever base is then pulled away from the bacterium at a constant velocity.

Movie S1. Cartoon illustrating the uncoiling of a pilus by an AFM tip. A mannose-covered AFM tip attaches to the terminal FimH subunit of a type I pilus. The cantilever bends as the pulling force on the pilus increases. When the force is sufficient, individual FimA subunits uncoil in discrete steps from the pilus shaft, consistent with the distance jumps in Figure 3B.

Tensile **toughness** is the amount of energy per unit volume that a material can absorb before rupturing, measured in units of joules per cubic meter ($J * m^{-3}$).

Tensile **strength** is the amount of force that a material can withstand before rupturing, measured in units of newtons per square meter ($N * m^{-2}$) or pascals.



Source: Forero M, Yakovenko O, Sokurenko EV, Thomas WE, Vogel V (2006) Uncoiling mechanics of *Escherichia coli* type I fimbriae are optimized for catch bonds. *PLoS Biol* 4(9): e298 PMID: 16933977.

Source: Björnham O, Axner O (2010) Catch-bond behavior of bacteria binding by slip bonds. *Biophys J* 99(5): 1331-1341 PMID: 20816044.

Source: Aberg V, Fällman E, Axner O, Uhlin BE, Hultgren SJ, Almqvist F *2007) Pilicides regulate pili expression in E. coli aithour affecting functional properties of the pilus rod. *Mol Biosyst* 3: 214-218 PMID: 17308668.

(A) Discrete coiling and uncoiling events of a single type I pilus. Schematic showing the structure of a Type I pilus attached to an AFM (atomic force microscope) cantilever tip. The lectin domain of the FimH adhesin binds to mannose on the AFM tip. The pilus tip consists of the FimH adhesin (comprising two domains: lectin domain plus pilin domain) linked to the FimG pilin monomer linked to the FimF pilin monomer. FimF is linked to the 7-nm-diameter pilus pilus shaft made from helically coiled FimA pilin subunits. Each FimA subunit contributes 0.7 nm to the length of the helical shaft. The length of each FimA subunit is approximately 5.7 nm.

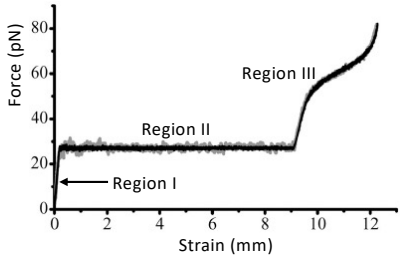
(B) Schematic of a single Type I pilus showing the "layer-to-layer" bonds (red lines) between adjacent turns of the helical structure, which stabilize the "uncoiled" helical structure. As the pilus is stretched, the layer-to-layer bonds are broken sequentially, one at a time, resulting in uncoiling of the helix. Each layer is stabilized by bonds linking it to the layer below and the layer above, with one exception: the layer at the "leading edge" of the boundary between the coiled and uncoiled regions is stabilized by only one set of layer-to-layer bonds linking it to the layer below but not to the layer above, which has already uncoiled. Consequently, bonds at the "leading edge" are much more vulnerable to breakage than bonds that are buried deeper in the helical structure, and so the helix uncoils, one layer at a time, only at the leading edge.

(C) Force-strain strain curve of a single pilus from an elongation measurement obtained with optical tweezers. The black curve represents a fit obtained using a computer simulation program. **Region I** of the force curve originates from elastic stretching of the helical structure of the pilus. **Region II** is due to sequential uncoiling of the layer-to-layer bonds (and thereby of the helical structure) of the pilus. **Region III**, with its characteristic wave-shape, is due to elastic stretching of the linearized pilin protein sequence, which is opposed by "head-to-tail" bonds between adjacent pilin subunits.

Does this non-linear force-strain behavior remind you of something we explored in an earlier lecture? Like intermediate filaments, maybe?

40

Type I pili are said to be “non-Hookean” because:

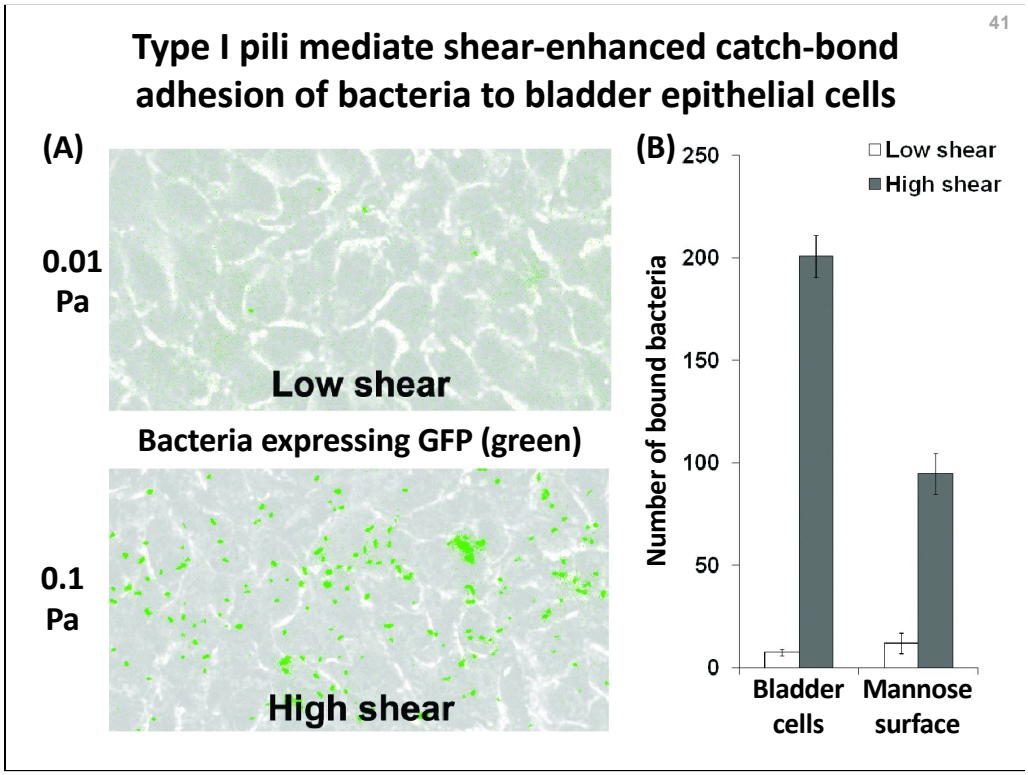


A. The relationship between the applied force and pilus elongation is linear (F = –*k* * D) for Type I pili.

B. A constant force is required for elongation in *Region II*.

C. The force required for elongation in *Region I* is greater that the force required for elongation in *Region II*.

Answer: (B)



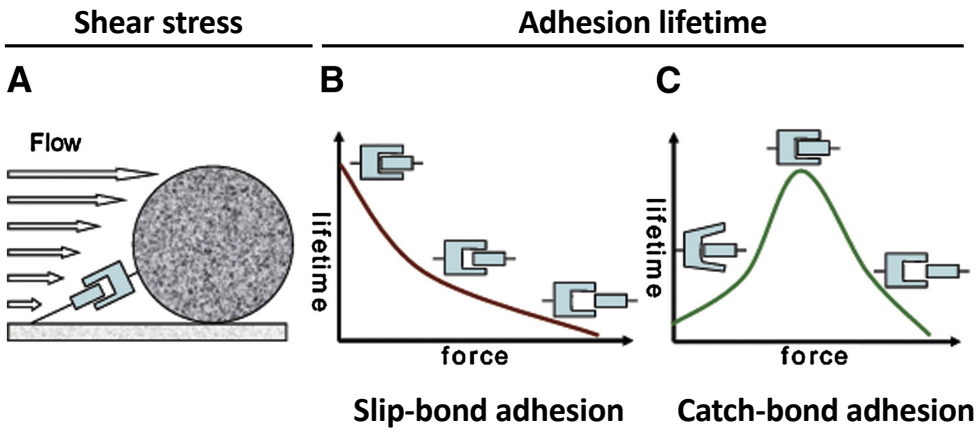
Source: Aprikian P, Interlandi G, Kidd BA, Le Trong I, Tchesnokova V, Yakovenko O, Whitfield MJ, Bullitt E, Stenkamp RE, Thomas WE, Sokurenko EV (2011) The bacterial fimbrial tip acts as a mechanical force sensor. *PLoS Biol* 9(5): e1000617 PMID:21572990.

Figure 2. **(A)** Binding of *E. coli* to bladder epithelial cells at low (0.01 Pa) and high (0.1 Pa) shear stress in a flow chamber. **(B)** Level of *E. coli* binding under low (0.01 Pa) and high (0.1 Pa) shear stress to bladder epithelial cells and a surface coated with mannose.

Newtons (N) in kg * m * s^{-2}

Pascals (Pa) in N * m^{-2} or kg * m^{-1} * s^{-2}

Type I pili mediate shear-enhanced catch-bond adhesion of bacteria to bladder epithelial cells

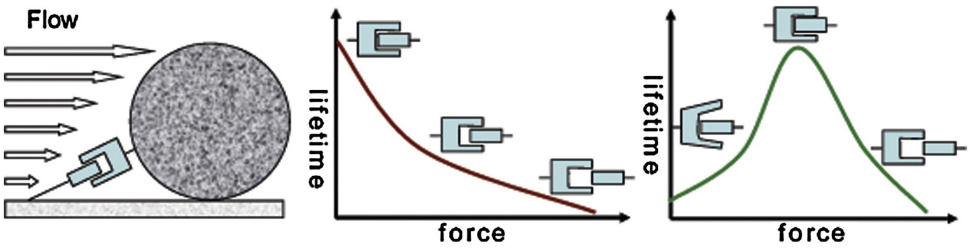


Source: Sokurenko EV, Vogel V, Thomas WE (2008) Catch-bond mechanism of force-enhanced adhesion: counter-intuitive, elusive, but ... widespread? *Cell Host Microbe* 4(4): 314-323 PMID: 18854236.

Figure 1. Shear stress and its effect on slip bonds or catch bonds. **(A)** Schematic presentation of shear stress on a cell adhering to a surface. **(B)** Dependence of the adhesion lifetime of receptor-ligand interactions on the force level in ***slip bonds***. **(C)** Dependence of the adhesion lifetime of receptor-ligand interactions on the force level in ***catch bonds***.

The full-in ligand configuration corresponds to strong binding (low probability of the bond dissociation). The half-in ligand or open receptor configurations correspond to weak binding (high probability of the bond dissociation).

"Catch-bond" adhesion means:



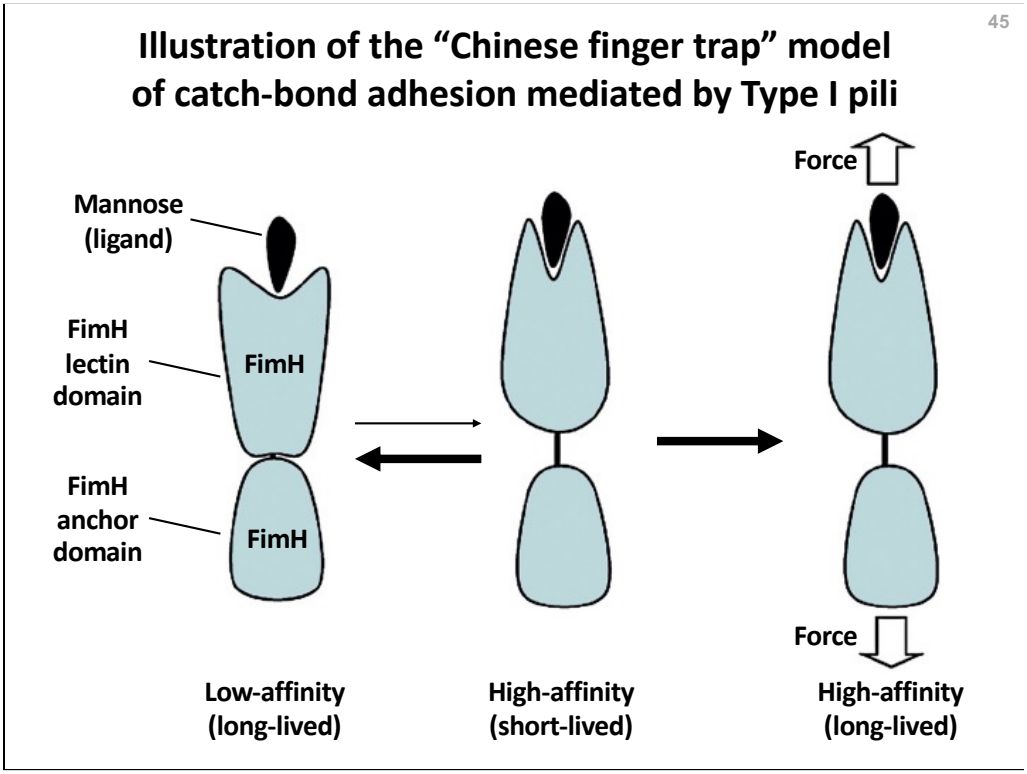
A. As the force acting on the pilus increases, adhesion strength decreases.

B. As the force acting on the pilus increases, adhesion strength increases.

C. As the force acting on the pilus increases, adhesion strength remains the same.

Answer: (B)

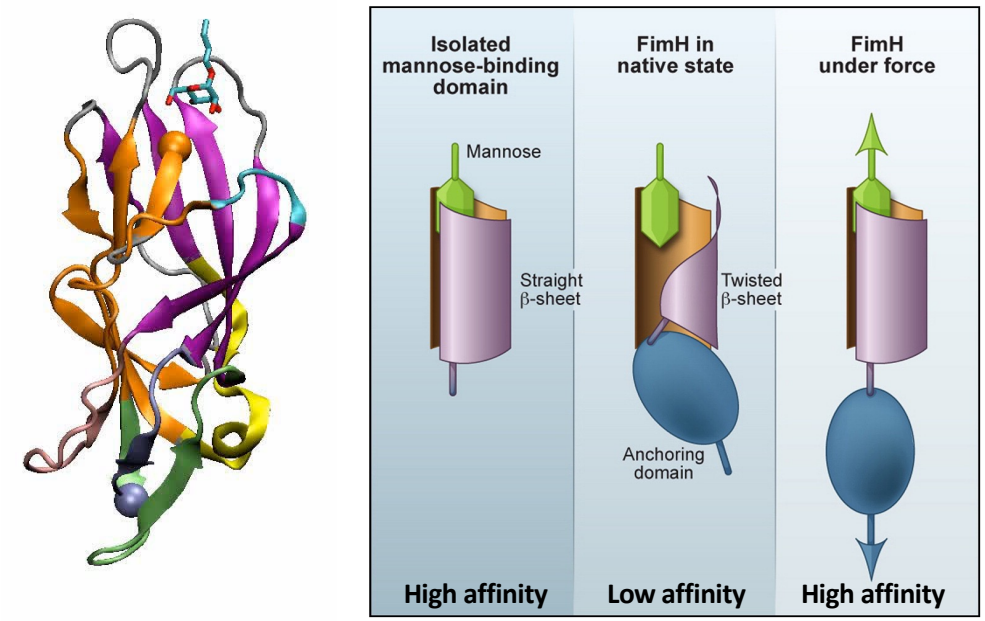
The “Chinese finger trap” toy is a model for catch-bond adhesion mediated by Type I pili



Source: Sokurenko, E.V., Vogel, V., Thomas, W.E. (2008) Catch-bond mechanism of force-enhanced adhesion: counterintuitive, elusive, but ... widespread? *Cell Host Microbe* 4(4): 314-323 PMID:18854236.

Schematic representation of putative allosteric conformational states of FimH. **(A)** The "open" (low-affinity binding) conformation, which is long-lived. **(B)** The "closed" (high-affinity binding) conformation, which is short-lived. **(C)** The "closed" (high-affinity binding) state is stabilized by tensile forces (vertical arrows).

Mechanical force changes the shape of FimH protein from "low-affinity binding" state to "high-affinity binding" state



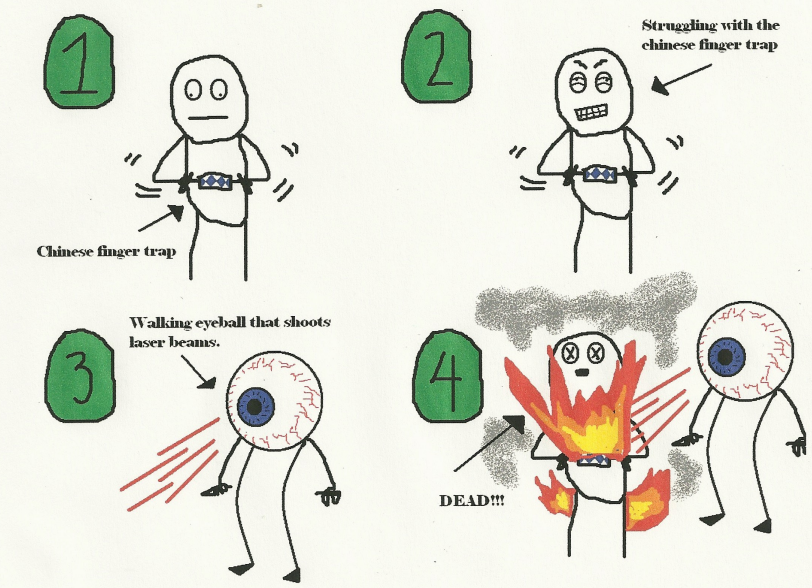
See: Movie_Slide46.mov posted on Moodle.

Source: Le Trong I., Aprikian P., Kidd B.A., Forero-Shelton M., Tchesnokova V., Rajagopal P., Rodriguez V., Interlandi G., Klevit R., Vogel V., Stenkamp R.E., Sokurenko E.V., Thomas W.E. (2010) Structural basis for mechanical force regulation of the adhesin FimH via finger trap-like beta sheet twisting. *Cell* 141(4): 645-655 PMID:20478255.

Left: Movie S1. The *Escherichia coli* type I pilus adhesive protein, FimH, mediates shear-dependent binding to mannosylated surfaces via force-enhanced allosteric catch bonds. This video shows the underlying structural changes causing this behavior. When force is applied and the auto-inhibitory anchor domain of FimH is pulled away, the mannose-binding lectin domain of FimH switches from a twisted compressed conformation to an untwisted elongated conformation, much like a Chinese finger-trap toy. The initial conformation shown here is the lectin domain (the first 158 amino acids) of the X-ray structure of FimH in the low-affinity compressed state. The final conformation contains the lectin domain in the high-affinity state.

Right: Schematic.

Illustration of the "Chinese finger trap"



Stuck in the trap? See: https://www.youtube.com/watch?v=xEa68NjZp0w

Stuck in the trap? Check out this instructional video: https://www.youtube.com/watch?v=xEa68NjZp0w

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Which of the following statements is true:

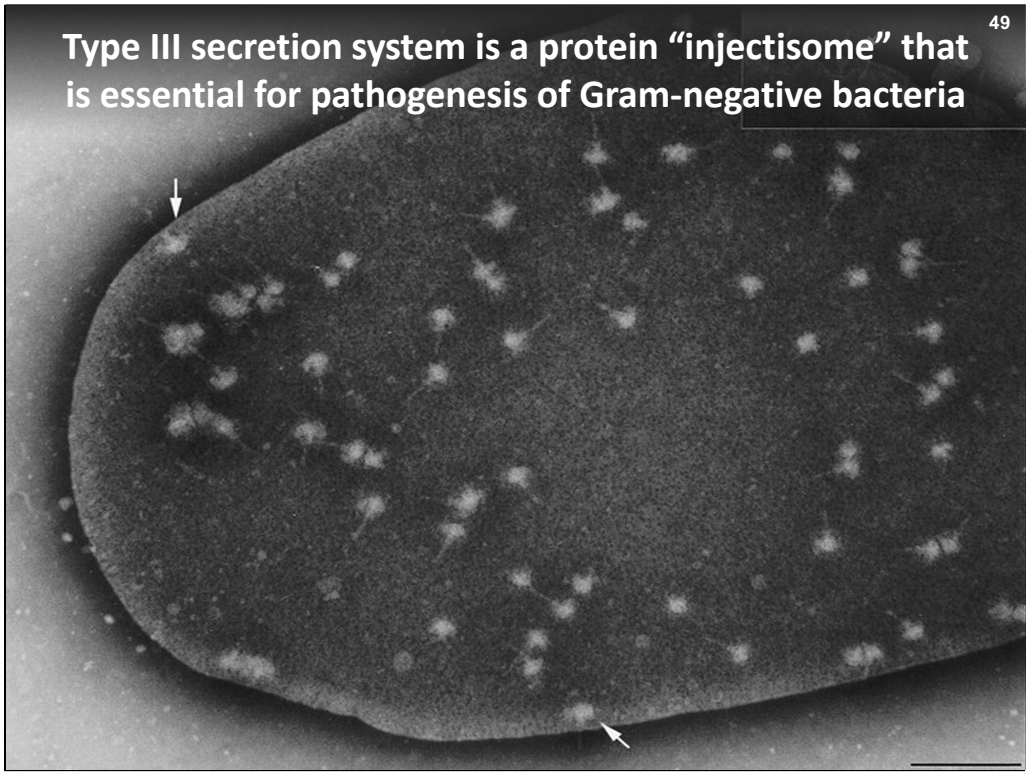
A. The force-strain relationship is linear for Type I pili and non-linear for Type IV pili.

B. The force-strain relationship is non-linear for Type I pili and linear for Type IV pili.

C. Type I pili display “slip-bond” adhesion.

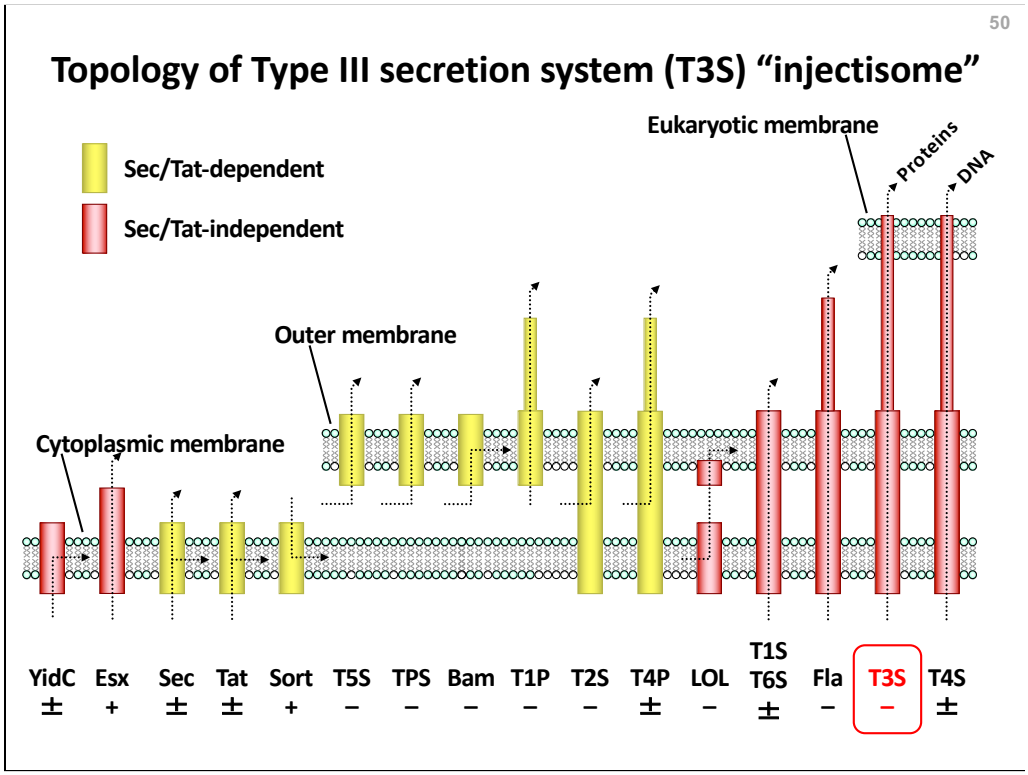
D. Type IV pili display “catch-bond” adhesion.

Answer: (B)



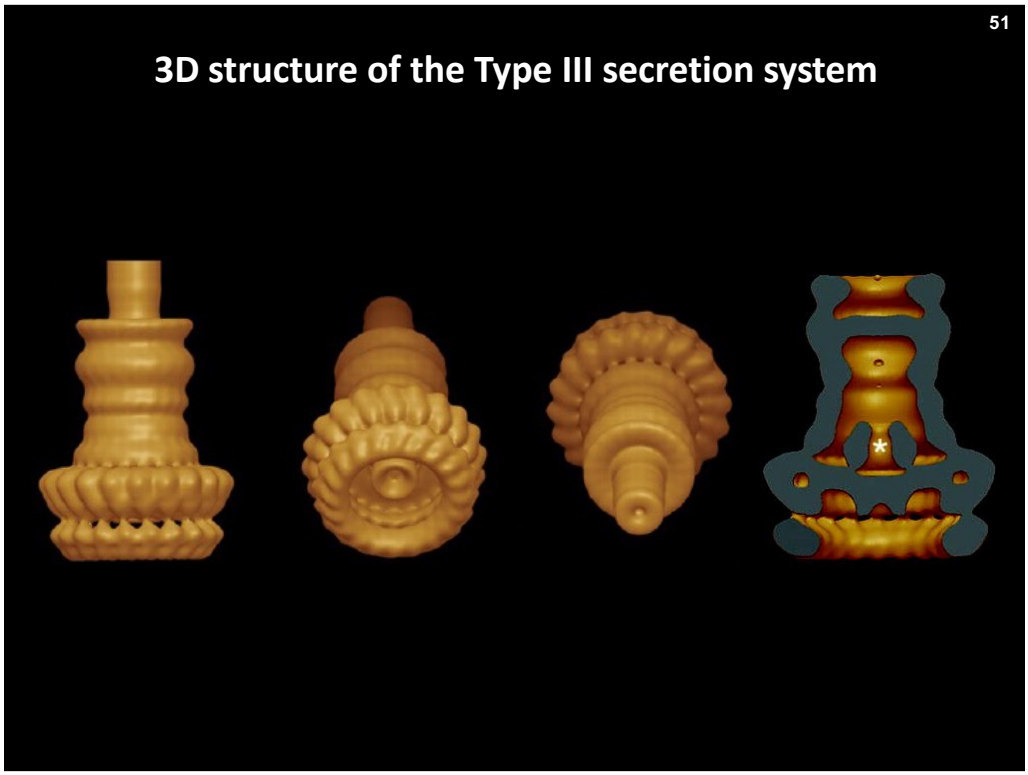
Source: Blocker A, Gounon P, Larquet E, Niebuhr K, Cabiaux V, Parsot C, Sansonetti P (1999) The tripartite type III secreton of *Shigella flexneri* inserts IpaB and IpaC into host membranes. *J Cell Biol* 147(3): 683-693 PMID:10545510.

Figure 5(A). Morphological identification and analysis of the type III secretion system assemblies of the human pathogen *Shigella flexneri*. Osmotically shocked and negatively stained wild-type *Shigella* which had been induced to turn on the type III secretion system with Congo red were visualized by electron microscopy. Arrows show the position of the type III secretion system at the margin of bacteria. The neck and bulb are inside the body of the bacterium while the needle is protruding outside the outer membrane. Inset shows the periphery of partially lysed bacteria. Only the protruding needle is clearly visible. The neck is poorly distinguished and the bulb is masked inside the body of the bacterium. With the method used here one can not assume that the two lines at the margin of the bacterium are its inner and outer membranes.



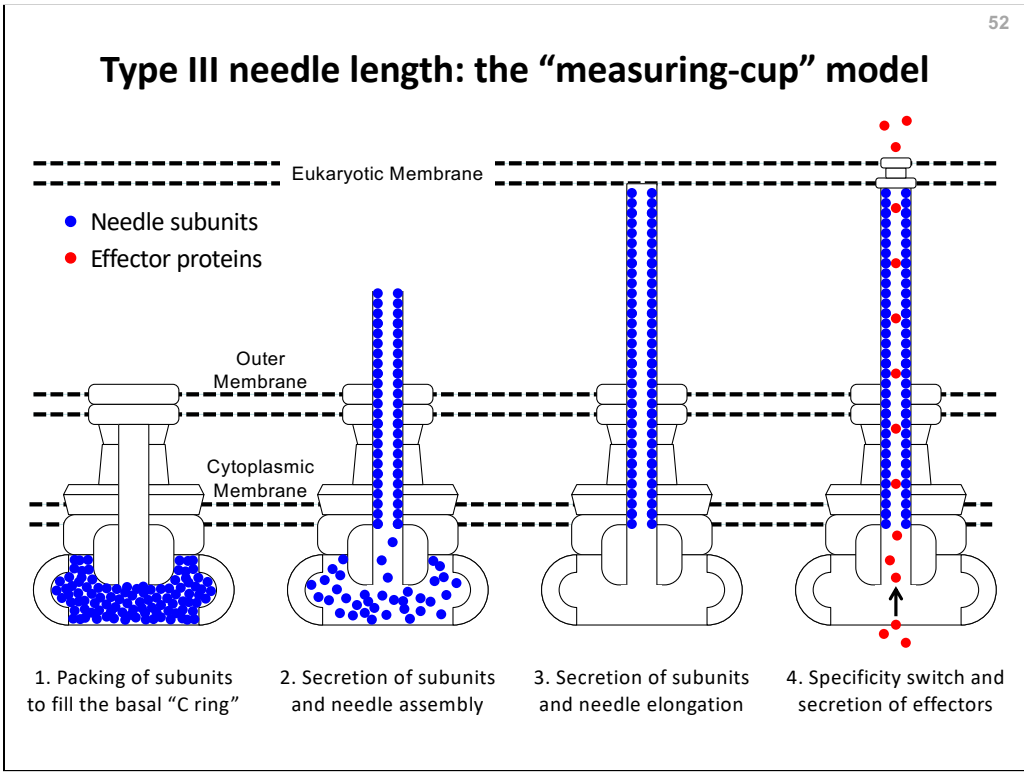
Source: Papanikou W, Karamanou S, Economou A (2007) Bacterial protein secretion through the translocase nanomachine. *Nat Rev Microbiol* 5(11): 839-851 PMID:17938627.

Note the topology: the base of the Type III secretion system “injectisome” crosses the cytoplasmic membrane, the periplasmic space, and the outer membrane, while the shaft of the pilus extends into the extracellular space and, at its tip, passes through the cytoplasmic membrane of the targeted eukaryotic cell. Thus, proteins secreted by the type III secretion system can travel directly from the cytoplasm of the bacterial cell into the cytoplasm of the eukaryotic cell.



Source: Marlovits TC, Kubori T, Sukhan A, Thomas DR, Galán JE, Unger VM (2004) Structural insights into the assembly of the type III secretion needle complex. *Science* 306(5698): 1040-1042 PMID:15528446.

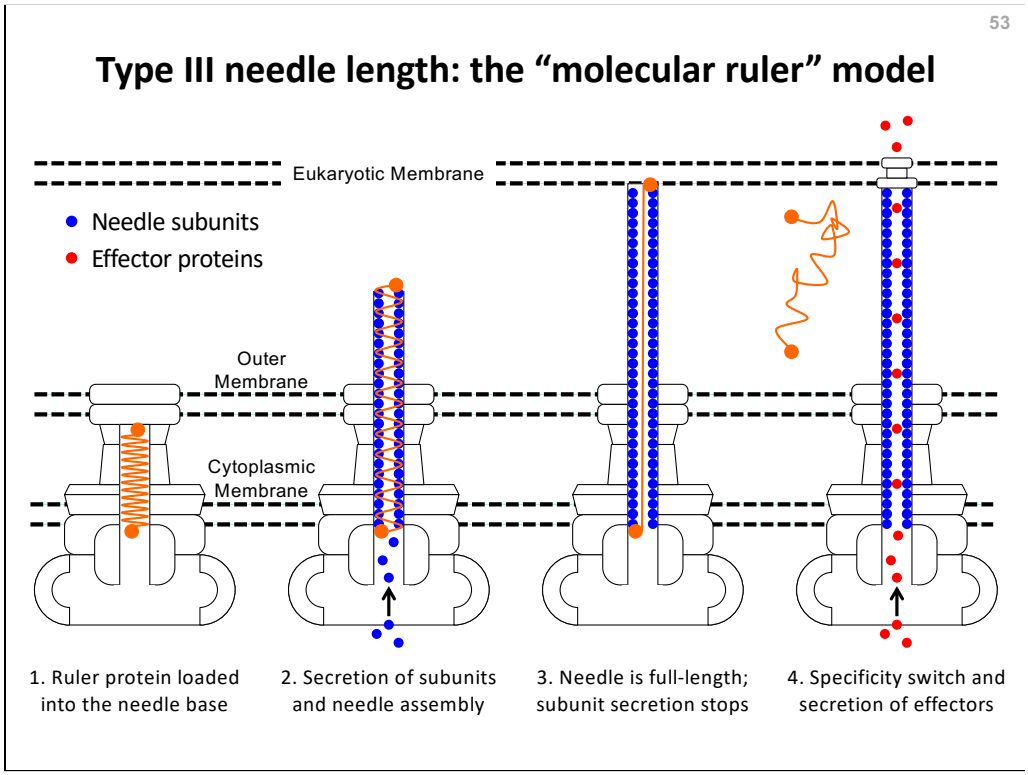
The three-dimensional structure of the Type III secretion system of *Salmonella typhimurium* (a Gram-negative bacterial pathogen that is a common cause of food poisoning in humans) was solved at a spatial resolution of 17 Angstroms using cyro-electron microscopy (Cryo-EM). For comparison: an alpha helix consisting of 20 amino acid residues is about 30 Angstroms in length. So a spatial resolution of 17 Angstroms is impressive for 2004, when this paper was published, and spatial resolution of Cryo-EM has only improved since then.



Source: Cornelis GR (2006) The type III secretion injectisome. *Nature Rev Microbiol* 4(11): 811-825 PMID: 17041629.

Figure 6(A). Control of the length of the Type III secretion system (T3SS) needle: the "measuring cup model". **(1)** First, the the basal structure (C-ring) is filled to capacity with needle subunits (blue dots). **(2)** The needle subunits are exported and the needle elongates. **(3)** When all of the subunits have been used up (i.e., when the C ring is empty), secretion of needle subunits stops and the specificity of the T3SS switches from needle subunits to effector proteins. **(4)** Effector proteins (red dots) are secreted into the cytoplasm of the targeted eukaryotic cell.

In this model, the size of the C-ring cup ("measuring cup") determines the number of needle subunits that will be assembled and, consequently the length of the needle. If the C-ring is small the needle will be short. If the C-ring is large the needle will be long.



Source: Cornelis GR (2006) The type III secretion injectisome. *Nature Rev Microbiol* 4(11): 811-825 PMID: 17041629.

Figure 6(B). Control of the length of the Type III secretion system (T3SS) needle: the "molecular ruler model". **(1)** The "ruler protein" (orange spring) enters the secretion channel after completion of the basal structure (C ring). **(2)** The needle subunits (blue dots) are exported and the needle elongates. Elongation of the needle causes the "ruler protein" to stretch. **(3)** When the "ruler protein" is fully stretched, further growth of the needle stops. **(4)** The fully stretched "ruler protein" triggers a switch in specificity of the T3SS channel from secretion of needle subunits to secretion of effector proteins. The ruler protein is then released and effector proteins (red dots) are secreted into the cytoplasm of the targeted eukaryotic cell.

In this model, the length of the "ruler protein" ("molecular ruler") determines the number of needle subunits that will be assembled and, consequently the length of the needle. If the "ruler protein" is short the needle will be short. If the "ruler protein" is long the needle will be long.

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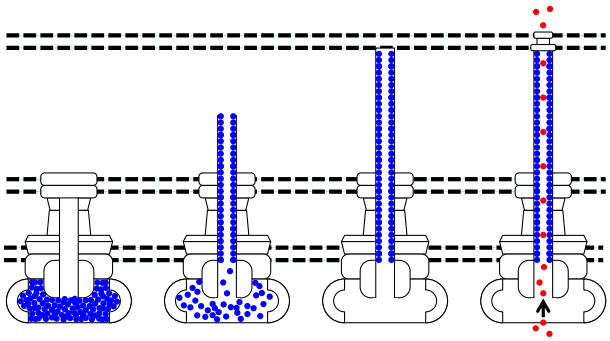
The “measuring cup” model of Type III needle length control predicts that needle length could be increased by:



A. Increasing the expression of needle subunits.

B. Increasing the length of the ruler protein.

C. Increasing the size of the basal “C-ring”.

D. Delaying the expression of effector proteins.

Answer: (C)

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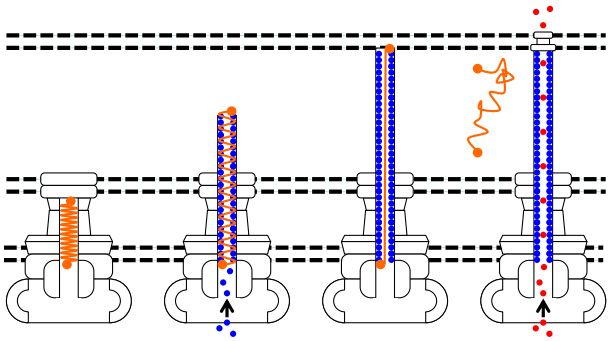
The “molecular ruler” model of Type III needle length control predicts that needle length could be increased by:



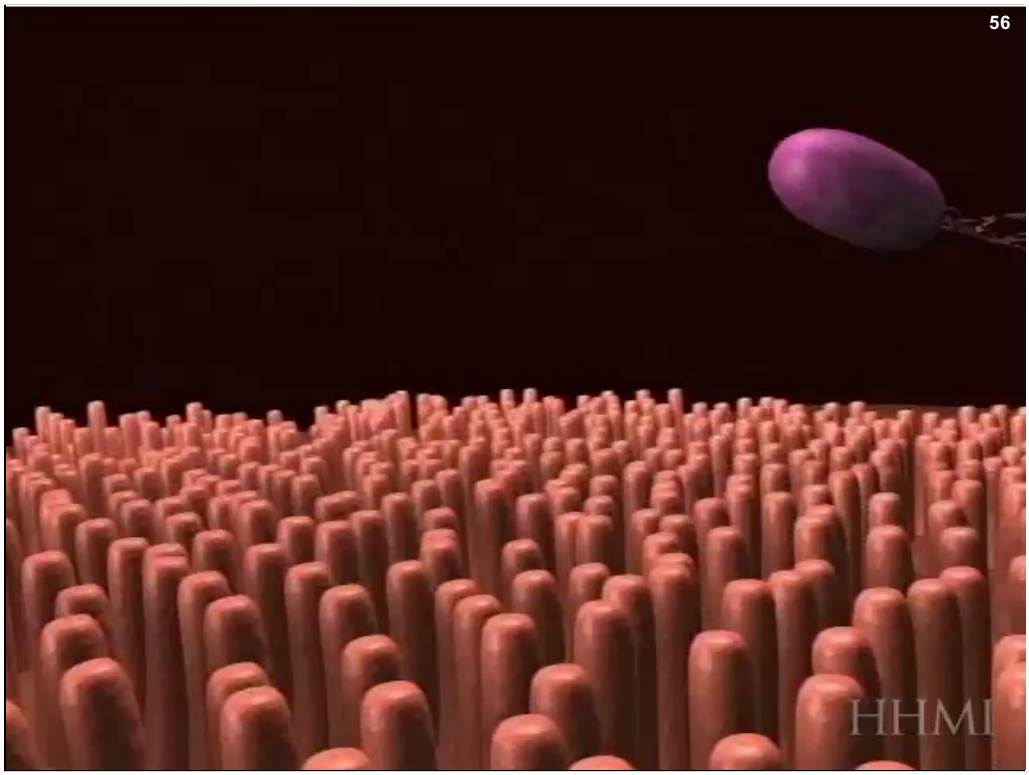
A. Increasing the expression of needle subunits.

B. Increasing the length of the ruler protein.

C. Increasing the size of the basal body C-ring.

D. Delaying the expression of effector proteins.

Answer: (B)



See: Movie_Slide56.mov posted on Moodle.

The surface of epithelial cells of the intestine is covered with microvilli, finger-shaped extensions of the cell that vastly increase the surface area for absorbing nutrients. In the animation, a single *E. coli* bacterium (purple) latches on to the surface of an intestinal epithelial cell (brown) using long, tether-like pili. Pili are made of strands of long protein filaments that can adhere to the microvilli on the surface of intestinal cells.

Once in contact with the bacterium, the microvilli disappear from a patch of the cell surface, the bacterium comes into closer contact with the intestinal cell surface, and the next phase in the infection process begins.

The tethered bacterium now uses a specialized injector system to deliver some of its own proteins into the cell that it is invading. The injector systems that bacteria use are fascinating and are composed of several different proteins. In this case a type III secretion system “injectisome” is used, which is specialized for pumping things into other cells. The bacterium uses the injector system, much like a syringe, to introduce several bacterial proteins into the intestinal cell that force it to cooperate in its own infection.

A needlelike tube (purple) called EspA projects from the bacterium to the intestinal cell surface. Now two proteins (green) named EspB and EspD travel through the tube to form an opening in the intestinal membrane through which additional bacterial proteins can move into the cell. With the tube and pore complete, the bacterium now injects a protein called Tir (red) into the cell. The Tir proteins insert themselves into the intestinal cell membrane. A portion of the Tir protein projects beyond the cell surface and binds to a protein on the bacterial cell surface called intimin (blue cups). Now the membranes of the intestinal cell and the bacterium are locked together, and the intestinal cell is in trouble. The Tir proteins become phosphorylated by intestinal cell proteins (blue balls). The stage is set for the next step, pedestal formation.

The bacterium is now firmly bound to the intestinal cell surface via the interaction between the Tir and intimin proteins. Pedestal formation, a very active and striking process, begins. Another intestinal cytoskeletal protein (orange booties) binds to a portion of the bacterial Tir protein that is inside the cell. Once these proteins bind, long strands of actin (yellow balls) start to form. The actin filaments build up directly beneath where the bacterium is bound to the intestinal cell. As the actin filaments lengthen, they push the cell membrane upward, and the bacterium becomes perched atop a dramatic pedestal formed by the intestinal cell. Once many enteropathogenic bacteria have adhered to the intestinal lining, symptoms of the infection (diarrhea) commence.