Inactive conformation enhances binding function in physiological conditions

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Many receptors display conformational flexibility, in which the binding pocket has an open inactive conformation in the absence of ligand and a tight active conformation when bound to ligand. Here we study the bacterial adhesin FimH to address the role of the inactive conformation of the pocket for initiating binding by comparing two variants: a wild-type FimH variant that is in the inactive state when not bound to its target mannose, and an engineered activated variant that is always in the active state. Not surprisingly, activated FimH has a longer lifetime and higher affinity, and bacteria expressing activated FimH bound better in static conditions. However, bacteria expressing wild-type FimH bound better in flow. Wild-type and activated FimH demonstrated similar mechanical strength, likely because mechanical force induces the active state in wild-type FimH. However, wild-type FimH displayed a faster bond association rate than activated FimH. Moreover, the ability of different FimH variants to mediate adhesion in flow reflected the fraction of FimH in the inactive state. These results demonstrate a new model for ligand-associated conformational changes that we call the kinetic-selection model, in which ligand-binding selects the faster-binding inactive state and then induces the active state. This model predicts that in physiological conditions for cell adhesion, mechanical force will drive a nonequilibrium cycle that uses the fast binding rate of the inactive state and slow unbinding rate of the active state, for a higher effective affinity than is possible at equilibrium.

conformational dynamics | receptor | ligand | adhesion | mechanical force

It is now known that few proteins recognize ligands through a lock and key mechanism, in which the binding pocket is in essentially the same conformation whether or not ligand is bound. Instead, the conformation of the binding pocket is usually dynamic. Conformational dynamics have many functions for receptors as well as enzymes, so for simplicity, we will use the term ligand to describe substrates and products as well as molecules that are not changed by binding. For allosteric proteins, an obvious function of conformational changes in the pocket is to allow regulation of ligand binding by an allosteric effector. For other proteins, flexibility in the binding pocket allows proteins to bind structurally distinct ligands (1), which may in turn facilitate evolution of new structures and functions (2, 3). In many cases, the inactive conformation of the pocket is relatively loose, and the active conformation tightens around the ligand, often due to the closing of a hinge (4) between two domains, or loops that are described as a gate (e.g., refs. 5–7) or a lid (e.g., refs. 8–11) because they close over the ligand in binding pocket. The ability of the pocket to close around different ligands can contribute to specificity (5). Switching to the inactive state of the receptor dramatically increases the dissociation rate, so pocket dynamics controls the residence time of ligands (4, 12), and thus the catalytic rate of most enzymes (3). The importance of the inactive state to association rates is less clear; inactive states have been shown have lower (4, 12), similar (13), or higher (14) association rates relative to the active state, and the functional importance of the differences is unclear.

We hypothesize that inactive states can have faster ligand association rates, and that this is important for nonequilibrium processes, where the kinetics, or rates, of individual steps are more important than overall affinity because the process does not reach equilibrium. An example of a nonequilibrium process is cell adhesion. Because adhesive receptors bind to ligands that are anchored to other cells or surfaces, the adhesive bonds are subjected to tensile mechanical force due to cytoskeletal contraction, fluid–flow–induced drag forces, or other stresses. This tensile force can only be applied between receptor and ligand after the bond forms, so binding and unbinding occur in different conditions, and are thus not occurring in a reversible manner as required to reach equilibrium. Many adhesive receptors form catch bonds (15–19), which are bonds that are activated by tensile force to become longer lived, so the influence of force on this binding process is nontrivial. The functional advantage of the inactive state in this nonequilibrium process is not known.

The Escherichia coli adhesin FimH is a model for addressing the role of the inactive state in the nonequilibrium process of cell adhesion, because FimH forms catch bonds that involve allosteric regulation (19, 20), like many other adhesive proteins (21–24) and the structural basis of allostery in FimH is well understood (20). The isolated mannose-binding lectin domain has an elongated regulatory region and a tightly closed binding site that binds α-methyl-mannose (αMM) with an affinity of ~1–2 μM (25, 26) and mannosylated BSA (man-BSA) with a lifetime of many minutes (20). However, FimH is normally incorporated into fimbrial adhesive organelles, and in this state, the naturally predominant variants such as K12 have a compressed regulatory region and a mannos-binding pocket that is 3 Å wider because the gate-like clamp loop is open (20). We refer to this as the inactive state.

Significance

The binding pocket of a receptor often switches between two conformations, with the tight “active” conformation binding ligand with higher affinity than the loose “inactive” one. We demonstrate here that in physiologically relevant nonequilibrium conditions, stabilizing the active conformation actually reduces binding. We furthermore show that ligand binds most rapidly to the inactive conformation, but detaches most slowly from the active conformation, so that each conformation provides an advantage to binding. Mechanical force regulates many adhesive receptors in a way that creates a nonequilibrium cycle that would take advantage of the best property of each conformation, creating a higher effective affinity than either state alone. This provides new insights into the importance of both the inactive state and nonequilibrium conditions.

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because K12 has an affinity of 300 μM for aMM (25). Most bonds between K12 and man-BSA last under 1 s, and a small fraction are much longer lived, demonstrating that K12 can bind to mannose in both the inactive and active states (20). The structure of the short-lived inactive bound state is not known, but mutational studies suggest weak, or sequential, allosteric coupling, in which the clamp loop closes transiently around mannose and the regulatory region remains compressed (27). FimH forms catch bonds because mechanical force induces the elongated state of the regulatory region, which stabilizes the closed form of the clamp loop (20, 28). Many variants of FimH have been either engineered or discovered in clinical isolates that increase binding to mannose in static conditions (27, 29, 30) by destabilizing the inactive state relative to the active state (27). Evolution selects against these mutations (30, 31), which strongly suggests that the inactive state provides a functional advantage in vivo, but the mechanism for this advantage remains unknown.

Here we compare FimH variants to determine whether and how the inactive state provides a functional advantage to cell adhesion in nonequilibrium conditions. We refer to K12 as wild-type FimH. The FocH variant has the same affinity for aMM as the isolated lectin domain (25), so we refer to FocH as activated FimH. Although activated FocH mediated more bacterial attachment than did wild-type K12 at low flow rates, it mediated less attachment at high flow rates. Indeed, for over a dozen FimH variants, the number of bacteria binding at high flow was inversely proportional to the amount of FimH in the active state, as determined by a conformation-sensitive antibody. We used atomic force microscopy (AFM) and surface plasmon resonance (SPR) to show that FocH and K12 have similar mechanical strengths, but that activated FocH has a much slower bond association rate. Together, these results demonstrate that the inactive state of FimH is necessary to initiate bacterial adhesion at high flow because it forms bonds quickly. This suggests that FimH binds through a novel kinetic-selection model in which ligand selectively binds to the inactive state due to its faster association rate, but then induces the active state, and that mechanical force creates a nonequilibrium cycle that combines the rapid binding of the inactive state with the slow unbinding of the active state for a higher effective affinity than that of either state at equilibrium.

**Results**

**Bacterial Adhesion in Flow.** To determine whether the inactive state of FimH is important for mediating adhesion in flow, we compared two strains of *E. coli* expressing the activated FocH variant of FimH, versus the wild-type K12 variant that is in the inactive state before binding. The two strains had an otherwise identical genetic background, and expressed similar levels of FimH when analyzed by flow cytometry (Table S1). First we performed binding assays, in which we infused bacteria at various shear rates over surfaces coated with man-BSA, and used video microscopy to count the number of bacteria bound to the surface. Man-BSA was used since *E. coli* adhesion to this glycan is similar to that on FimH-mediated bacteria expressing K12 displayed shear-enhanced adhesion (Fig. 1B). Even at 1 Pa, the highest shear stress studied in Fig. 1A, both strains of bacteria to the surface at 0.035 Pa and then increased flow stepwise while tracking bound bacteria to measure movement or detachment (Fig. 1B). Even at 1 Pa, the highest shear stress studied in Fig. 1A, both strains of bacteria remained bound without even moving. Indeed, all bacteria remained bound through 10 Pa, and the fraction that moved at 3 and 10 Pa was similar for the two FimH variants. This demonstrates that the inability of the activated FocH variant to mediate binding at moderate to high flow does not reflect an inability to remain attached.

Because binding of bacteria from solution requires the ability to initiate attachment as well as to remain attached, we next measured initial attachment rates from the original binding videos by counting each bacterium when it first appeared on the surface, whether it then remained bound for less than a second, rolled along the surface, or stuck firmly to the surface. At the two lowest shears measured, the bacterial attachment rate for K12 could not be accurately measured because many bacteria in these conditions appeared in only one frame, suggesting that many more may have appeared for too short a time to detect. However, in conditions where initial attachment could be accurately measured (Fig. 1C), K12 mediated much more initial attachment than did FocH. Thus, the poor performance of the activated FocH variant at moderate to high flow can be explained by a deficiency in initiating bacterial attachment.

**Bond Kinetics and Mechanics.** To further understand the difference in bacterial binding mediated by the two variants, we investigated the difference in association and dissociation of FimH-mannose bonds for the two variants. First, AFM was used to study the mechanical strength of single bonds between mannose and FimH. An AFM cantilever was incubated with man-BSA. A tissue culture polystyrene surface was incubated with 0.01 mg/mL fimbrial tips, each of which contain a single FimH subunit (33). The cantilever was pressed to the surface for 10 s to allow bonds to form, and then retracted at a nominal rate of 1,000 pN/s until a sudden decrease in force indicated bond rupture (Fig. 2A). Bonds were observed in 15–20% of pulls, so 8% were multiple bonds if bond formation follows a Poisson distribution (34). We removed 8.6% from analysis because they showed multiple ruptures, so the final analyzed data should reflect only single bonds. For negative controls, 1% aMM was included in the buffer to block FimH from binding to man-BSA. The rupture forces were binned and presented as histograms in Fig. 2B. When FimH was not inhibited, both K12 and FocH formed bonds with man-BSA that ruptured primarily between 120 and 180 pN, with a peak at 150 pN. This characteristic rupture force was not observed in the negative controls, and is a known signature of single FimH-mannose bonds (19, 35). Thus, single FimH-mannose bonds...
Relative association rates of FimH-mannose bonds. (A) Effect of contact time on the frequency of specific bond formation in AFM experiments for FocH and K12 fimbrial tips binding to man-BSA. Specific bonds are defined as all ruptures between 120 and 180 pN, with aMM controls subtracted. Data shows mean and SD of two experiments. Dashed lines show the initial slopes, which provide an estimate of the rates of bond formation. Similar slopes for the two dates are shown as dashed and solid lines, providing an estimate of the relative rates of bond formation.

(Fig. 3) A typical AFM curve. (B) Rupture force histograms for binding of the indicated FimH in fimbrial tips to man-BSA, without and with aMM, which blocks specific adhesion to provide a negative control. Data shows mean and SD of two experiments.

Role of Conformational State in Bacterial Binding. To verify the importance of the inactive state in initiating adhesion at high flow, we expressed nine FimH variants sequenced from clinical isolates of E. coli and eight engineered FimH variants, all in isogenic E. coli strains. We characterized FimH expression using flow cytometry. Although there was some variation between strains (Table S1), these small differences were not well correlated with any of the other measurements we describe below \((R^2 < 0.41)\). We chose a selection of variants that cover a wide range of static binding strengths, as indicated by the ratio of the number of bacteria binding to monomannose vs. trimannose ligands (Table S1). This ratio, hereafter called the 1M/3M ratio, provides a normalized measurement of bacterial binding to man-BSA. We characterized FimH expression using flow cytometry. Although there was some variation between strains (Table S1), these small differences were not well correlated with any of the other measurements we describe below \((R^2 < 0.41)\). We chose a selection of variants that cover a wide range of static binding strengths, as indicated by the ratio of the number of bacteria binding to monomannose vs. trimannose ligands (Table S1). This ratio, hereafter called the 1M/3M ratio, provides a normalized measurement of bacterial binding to man-BSA (27).
To characterize the conformational state of each variant, we characterized purified fimbiniae in an ELISA with the Mab21 monoclonal antibody, which only recognizes the active state of FimH (37). Mab21 recognized the activated FocH variant rapidly, reaching half-maximal binding in 30 s (Fig. S2A). In contrast, Mab21 recognized a small fraction of the K12 variant at 30 s, and then recognition increased slowly over 120 min (Fig. S2B). This demonstrates that a small fraction of K12 is initially in the active state, and the rest activates slowly over time either spontaneously or due to the presence of Mab21. Either way, the binding to Mab21 at 30 s reflects the amount of a variant that is initially in the active state without mannos. Because FocH is primarily in the active state (25), the ratio of Mab21 binding at 30 s for each strain relative to FocH (hereafter called the Mab21 ratio) thus indicates the fraction of FimH that is in the active state (Table S1). By this measurement, 6 ± 6% of K12 was in the active state. For the full set of variants, the ability of bacteria to bind man-BSA in static conditions, as detected by 1M3M, roughly correlated with the fraction of FimH in the active state, as detected by the Mab21 ratio (R² = 0.89; Fig. S3).

We then measured the number of bacteria that bind to a man-BSA surface after 3 min at low (0.013 Pa) and at high (0.14 Pa) flow, for each of the 17 strains (Table S1). Although the relationship was weak, the two measurements were inversely correlated (R² = 0.63; Fig. S3), demonstrating that there is a general trade-off between low- and high-shear adhesion, but neither provides a clear prediction of the other. We also attached each strain at 0.035 Pa, then washed at 2 Pa for 2 min and 5 Pa for 1 min. None detached, and the fraction that remained stationary at 5 Pa was not correlated to the number binding at 0.14 Pa (R² = 0.2; Fig. S3). However, binding at low shear was fairly well correlated to the Mab21 ratio (R² = 0.85; Fig. S4), demonstrating that the active state is important for binding in low shear conditions. Binding at high-shear showed a biphasic relationship to the Mab21 ratio (Fig. 5B). Variants with extremely low ratios had slightly lower binding, likely because they activate poorly even under force. For the rest, the number bound at high shear was strongly inversely correlated with the Mab21 ratio (R² = 0.96; Fig. 5B), and thus the fraction of FimH in the active state. This demonstrates that E. coli binding to man-BSA via FimH at high shear stress (e.g., >0.1 Pa) requires the inactive state of FimH.

**Discussion**

Here we showed that a FimH variant called FocH that is in the active state bound more poorly to a surface at high flow, relative to a wild-type FimH variant called K12 that is primarily in the inactive state. The difference in high flow binding did not reflect a difference in the mechanical strength of the two variants because single bonds formed by both variants withstood the same force, and bacteria expressing both variants withstood similar flows. Instead, the difference reflected lower attachment rates by bacteria expressing FocH versus K12. The difference in initial bacterial attachment can be explained by a difference in bond association rates, as measured in both AFM and SPR, with K12 forming bonds 23-fold more quickly. For 17 FimH variants, the number of bacteria binding at high flow was inversely correlated with the fraction of FimH in the active state. Together, these data demonstrate that the inactive state of FimH forms bonds more quickly and is needed for bacteria to initiate binding at high flow.

Cells can benefit from any mechanism that helps initiate binding in moderate to high flow. FimH allows E. coli to colonize the mouth where shear stress due to salivary flow is estimated at 0.08 Pa (38), and the intestines where shear stress due to peristalsis is estimated at 1 Pa (39, 40). Our data showing the superior binding of wild-type K12 over activated FocH in this flow range (Fig. 1) can therefore explain the evolutionary dominance of variants like K12 that are in the inactive state. The need to bind in this shear regime is not unique to FimH and E. coli. Many pathogens as well as blood cells must bind in these and other niches that involve moving fluid, such as venous and arterial blood vessels, which experience a wall shear stress of 0.1–5 Pa (41). Although flow increases the effective attachment rate of macromolecules by replenishing the depleted layer near the binding surfaces (42, 43), high flow inhibits adhesion of cells (44), even when moderate flow enhances adhesion (32, 45). Drag force is often assumed to explain the reduced adhesion at high flow, but although 0.1-Pa shear stress may create enough drag force to shorten significantly bond lifetimes on larger eukaryotic cells (46), drag force on E. coli at 0.1 Pa is only 1.6 pN (47), which is much too low to break FimH bonds (20). A more likely reason for reduced cell adhesion at moderate to high flow is that hydrodynamic lift reduces the concentration of microparticles near the surface as flow increases (48, 49). Hydrodynamic lift should affect both bacteria and eukaryotic cells, which are microscale particles, but not nanoscale macromolecules. Although in vitro devices are usually oriented to take advantage of gravity to force the cells toward the surface, which can reduce or even eliminate the effect of hydrodynamic lift, this is not the case in vivo. It therefore makes sense that cells that must adhere in flow in vivo have evolved mechanisms to increase bond association rates.

These same fast rates will also enable cells to roll along a surface instead of detaching when high drag force shortens bonds lifetimes. Many adhesive receptors that mediate rolling adhesion in flow have also been shown to have inactive states (46), including von Willebrand factor (45), selectins (50), and integrins (21). Rolling cells expressing active high affinity variants of P-selectin detached as much (51) or more (50) than cells expressing wild-type P-selectin. SPR data on both activated variants appear to show lower association rates than data on wild-type P-selectin, but this was only confirmed quantitatively when the data were fit with a two-state model (51), not with the one-state model (50), likely because the latter did not kinetically fit the data. Our conclusions demonstrate the need to accurately determine kinetic as well as thermodynamic parameters to understand the importance of the inactive state in cell rolling.

We propose a novel kinetic-selection model, in which the inactive state is important to initiate binding. We hypothesize that this model may apply to all bonds with gates (5–7) or latches (8–11) that close over the ligand in the active state, as observed for FimH (Fig. 6A). As illustrated in Fig. 6B, the kinetic-selection model hypothesizes that the inactive (R) and active (R*) state may coexist before binding, but that ligand selects for the inactive state due to a faster association rate (kₐ > k₋ₐ), and then induces the active state (R → R⁎ → R⁎*). This model is distinct from the common conformational selection model (Fig. 6C), which also assumes that both states coexist before binding, but requires that ligand selects the active state, rather than the inactive state (R → R* → R⁎*). The kinetic-selection model is distinct from the common induced-fit model (Fig. 6D), which

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**Table 1. Rate constants and other parameters discussed in the paper**

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<th>Parameter</th>
<th>Value</th>
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<td>SPR for FocH</td>
</tr>
<tr>
<td>k⁻ₐ</td>
<td>1.3 × 10⁹ M⁻¹ s⁻¹</td>
<td>SPR for FocH</td>
</tr>
<tr>
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<td>1.3 × 10⁹ M⁻¹ s⁻¹</td>
<td>SPR for K12</td>
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<td>k₋₁₂</td>
<td>1.0 nM</td>
<td>k⁺₀/k₋₀</td>
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also requires that binding occurs through the inactive state ($R \rightarrow RL \rightarrow RL^*$), but assumes that this occurs because the active state does not exist before binding, not because of a difference in rate constants. The kinetic-selection model may be considered a mixture or variation of these common models, and like these, other models for conformational dynamics predicts virtually by definition that stabilizing the active state will increase affinity.

However, the kinetic-selection model predicts a functional advantage not predicted by the other models in nonequilibrium conditions such as those found during cell adhesion. The kinetic-alternative-conformation model alone predicts that chemical or mutational stabilization of the active state will dramatically reduce binding before reaching equilibrium, thus explaining the countervailing results of Fig. 3. Moreover, because mechanical stabilization of the active state can only occur after the complex forms, this disadvantage of the active state is completely bypassed during the nonequilibrium cycle that is driven by force, as illustrated in Fig. 6E. That is, ligand binds quickly to the inactive state ($R \rightarrow RL$), allowing mechanical force to induce the active state ($RL \rightarrow RL^*$) and prevent inactivation, so unbinding must occur through slow dissociation from the active state ($RL^* \rightarrow R^*$), followed by inactivation ($\rightarrow R$) now that ligand and force are gone. This nonequilibrium cycle creates a higher effective affinity than is possible for either state without mechanical force, by combining the fast association rate $k_{\text{on}}$ of the inactive states with the slow dissociation rate $k_{\text{off}}$ of the active state. Although the $k_{\text{on}}$ (inverse affinity) of the K12 and FocH variants for man-BSA in the SPR fits is 490 and 23 nM respectively, the effective $k_{\text{on}}$ of the K12 variant under force is $k_{\text{on}} \rightarrow RL^* \approx 1.0$ nM. This raises the question as to whether the effective affinity of other receptors is increased through nonequilibrium cycles involving kinetic selection. Although the cycle can be driven by mechanical force for adhesive receptors, it might also be driven by the chemical energy of downstream signaling events such as autophosphorylation for signaling receptors, so this concept should be considered for all receptors with conformational dynamics in the pocket, not just adhesive proteins exposed to mechanical force.

It should be noted that the kinetic-selection model does not make any assumptions about the internal structural dynamics of the receptor. The model should be equally valid whether there is an allosteric or localized conformational change in the receptor, and whether the change occurs in a single concerted step, or in sequential steps with one or more intermediates, as suggested for FimH (27, 52). For the kinetic-selection model, like the induced-fit model, the only requirement about the internal conformational dynamics of the receptor is that it be able to bind the ligand, at least transiently, in some form of the inactive state.

**Materials and Methods**

**Reagents.** Man-BSA was obtained from V-LABs, Inc. All other chemicals were obtained from Sigma. Recombinant strains were constructed using a fim null K12 derivative, AAEC191A as described previously (53). Fimbrial components were purified as described previously (37).

**Flow cytometry analysis.** Flow cytometry analysis was performed to determine FimH expression levels for the different strains, using Mab21 in the presence of 1% aMEM and Alexa Fluor 488-labeled goat anti-mouse IgG (Molecular Probes). Because aMEM stabilizes the active state of FimH, Mab21 probes the total amount of FimH rather than its state of activation in these conditions (37).

**ELISA.** ELISA with Mab21 were performed for immobilized fimbrin as described previously (37) except that Mab21 was incubated for the time indicated in each experiment. To calculate the Mab21 ratio, the optical density after 30-s

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**Fig. 5.** Importance of FimH conformation to function. The number of bacteria bound at (A) low 0.013-Pa shear stress and (B) high 0.14-Pa shear stress were normalized to the highest binding strain at each shear, and plotted against the Mab21 ratio (the fraction of FimH in the active state) for the K12 variant under different conditions. Data shows mean and SD of 2-3 experiments. The K12 variant is indicated by a gray triangle and FocH by a black square. The correlation between binding and Mab21 ratio is shown with the $R^2$ statistic for a linear fit. In B, the variants with lowest Mab21 ratio (black X’s) were excluded from the fit because the relationship was biphasic.

**Fig. 6.** Models for conformational dynamics. (A) The inactive state of FimH with mannose (orange) docked into the pocket (20), and the active state crystallized with mannose (26). A gate consisting of residues 1, 140 and 142 (cyan), closes around ligand in the active state. (B–E) Models are compared; the transitions and states that control model behavior are shown in black and those that are assumed to occur too slowly or rarely to affect model behavior are shown in light gray. In these models, a short wide and a long thin shape are used to illustrate the inactive and active states, respectively, independent of ligand binding. This is intended only to help the reader distinguish the inactive from the active states, rather than to reflect any assumption about the conformation of the various low-energy or transient states. (B) The kinetic selection model predicts that all four states coexist, but the active state binds and unbinds very slowly. (C) The conformational selection model assumes that ligand binds selectively to the active conformation. (D) The induced-fit model assumes that the active unbound conformation does not exist. (E) In the kinetic-selection model with mechanical force, the active complex inactivates much more slowly than it unbinds, switching which rate constant determines model behavior.
incubation with MatB21 for each strain was divided by that for Foch, to estimate the fraction of each strain in the active state.

Dynamic Adhesion. Binding, initial attachment, and detachment of E. coli were measured in flow. All bacteria were tested with surfaces prepared in an identical manner; each was incubated with 0.2 mg/mL man-BSA as described before (32). The shear stress used is noted in each case.

SVM was performed as described previously with a BiaCore 2000, using man-BSA as the immobilized ligand and fimbriae as the analyte (25). Molar concentrations were calculated from total protein concentrations assuming an average molecular mass of 15,000 kDa, which assumes average 1,000 FimA subunits per fimbrae. Model fits were performed using BioEvaluation software, with RI set to zero because any change in refractive index was subtracted out using a control channel that lacked mannose on the BSA. A one-state binding model model was used for Foch because this variant was suspected to be locked in the active state. A two-state binding model was used for K12 because this variant is known to switch states (19). For the K12 fit, the RMS was restricted to be similar to that for Foch because the low affinity of K12 meant the model would be under-determined without this restriction, and the restriction is justified because the same amount of man-BSA was immobilized on the chip in both sets of injections, the average mass of the two types of pili is the same.

AFM. Measurement of rupture forces was performed as described previously (19), except that 0.01 mg/mL fibrinial tips were incubated with the surface for both strains, and loading rate and contact time were varied as indicated in the figures. We use a small compressive force of 50 pN (Fig. 2A) during the contact, which is not expected to denature FimH because FimH functions well for 20 s at 50 μM of tensile force (20).