Catch-bond mechanism of the bacterial adhesin FimH

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Ligand–receptor interactions that are reinforced by mechanical stress, so-called catch-bonds, play a major role in cell–cell adhesion. They critically contribute to widespread urinary tract infections by pathogenic Escherichia coli strains. These pathogens attach to host epithelia via the adhesin FimH, a two-domain protein at the tip of type I pili recognizing terminal mannoses on epithelial glycoproteins. Here we establish peptide-complemented FimH as a model system for fimbrial FimH function. We reveal a three-state mechanism of FimH catch-bond formation based on crystal structures of all states, kinetic analysis of ligand interaction and molecular dynamics simulations. In the absence of tensile force, the FimH pilin domain allosterically accelerates spontaneous ligand dissociation from the FimH lectin domain by 100,000-fold, resulting in weak affinity. Separation of the FimH domains under stress abolishes allosteric interplay and increases the affinity of the lectin domain. Cell tracking demonstrates that rapid ligand dissociation from FimH supports motility of piliated E. coli on mannosylated surfaces in the absence of shear force.
Cell–cell adhesion often occurs under dynamically varying conditions and mechanical stress. In many cell–cell adhesion systems, the lifetime of adhesion–receptor complexes is increased under tensile mechanical force via ‘catch-bonds’, which permit capture or retention of cells under flow conditions while still allowing for release under reduced mechanical force. Catch-bond interactions are prominent in vascular systems and are formed, for example, by selectins for leucocyte recruitment\(^1,2\), by cadherins controlling tissue integrity\(^3,4\) in the epithelial adhesion of cancer cells\(^5\) and by the interactions between T-cell receptors (TCRs) and peptide-bound major histocompatibility complexes (MHC) on antigen-presenting cells\(^6,7\). Catch-bonds also play a major role in bacterial adhesion and infection by uropathogenic *Escherichia coli* strains, which are responsible for the vast majority of urinary tract infections (UTIs) in humans\(^8\). A first critical step in the establishment of infection is bacterial adhesion to urothelial cells under flow conditions, which is mediated by 0.1–2 μm long, proteinaceous filaments on the bacterial surface termed type 1 pilus\(^9,10\). Type 1 pilus are composed of up to 3,000 copies of the proteinaceous filaments on the bacterial surface termed type 1 binding pocket and rearranged swing, linker and insertion loops. Affinity about two orders of magnitude higher than that of full-length FimH prevents the interactions between FimHL and FimHP (ref. 24). FimH and the pilus assembly chaperone FimC, where FimC major histocompatibility complexes (MHC) on antigen-presenting cells\(^8,9\). Owing to its importance in establishing infection, FimH is an attractive target for the development of anti-adhesive drugs for UTI treatment\(^14,15\).

FimH is a two-domain protein, composed of an N-terminal, mannoside-binding lectin domain (FimH\(_L\)) and a C-terminal pilin domain (FimH\(_P\)). FimH\(_P\) possesses an incomplete immunoglobulin-like fold that is completed by insertion of an N-terminal donor strand of FimG, the subsequent subunit in pilus assembly\(^11\). The two-domain architecture of FimH is a prerequisite for catch-bond formation because the interactions between FimH\(_L\) and FimH\(_P\) determine the conformational state and ligand-binding properties of FimH (refs 12,16,17). A ‘compressed’ FimH\(_P\) conformation was observed in the crystal structure of FimH in the context of the type 1 pilus tip fibrillum in the absence of ligands, with an open binding site and interactions to FimH\(_P\) mediated via three loop segments: the swing (amino acids (aa.) 27–33), linker (aa. 154–160) and insertion loops (aa. 112–118)\(^11\). In contrast, an ‘extended’ FimH\(_P\) conformation was observed in crystal structures of the isolated, ligand-bound FimH\(_P\) domain\(^18-23\) and in the complex between FimH and the pilus assembly chaperone FimC, where FimC prevents the interactions between FimH\(_L\) and FimH\(_P\) (ref. 24). This extended form of FimH\(_P\) is characterized by a closed ligand-binding pocket and rearranged swing, linker and insertion loops.

Notably, isolated FimH\(_P\) was reported to show a ligand-binding affinity about two orders of magnitude higher than that of full-length FimH in the tip fibrillum\(^7,24\). Together with mutagenesis experiments disrupting the interdomain interface\(^26\), these data indicated that ligand-binding is linked to domain separation in FimH, and that mechanical force shifts the ligand-binding affinity towards that of the isolated FimH\(_P\). However, fundamental aspects of the mechanism underlying the force-dependent binding of FimH remained unknown: (i) How is domain-associated, full-length FimH interacting with ligands? (ii) Does ligand-binding directly induce domain separation? (iii) How are interdomain interactions linked to the ligand-binding affinity of FimH and the kinetics of ligand-binding and dissociation? To address these questions, we designed a stable, soluble variant of full-length FimH that is equivalent in its structural and functional properties to those of FimH in the assembled filmbrial tip. This variant allowed us to obtain high-resolution structural snapshots of all functional states of FimH and to obtain a complete characterization of ligand-binding kinetics in solution. Together with molecular dynamics simulations, these data reveal a three-state mechanism of FimH catch-bond formation. FimH\(_P\) accelerates ligand release from FimH\(_L\) via dynamic allostery by 100,000-fold. In addition, using single-cell tracking experiments, we show that the modulation of ligand affinity by FimH\(_P\) is not only required for adhesion under mechanical stress, but also for efficient bacterial surface motility in the absence of shear force. Our results provide a first complete structural and kinetic description of a catch-bond system and establish a framework for the analysis of the distinct catch-bond mechanisms in other systems, which also commonly couple interdomain interactions to ligand affinity.

**Results**

**Construction of a peptide-complemented FimH.** Isolated FimH with its non-complemented pilin domain is only marginally stable and shows aggregation tendency under physiological conditions\(^27\). To establish a stable, isolated FimH molecule with all properties of FimH in the tip fibrillum, we complemented FimH\(_P\) with the donor-strand peptide of FimG (FimG residues 1–14; termed DsG). The FimH–DsG complex was obtained in good yields and purified after an *in vitro* reaction, mimicking the first donor-strand exchange (DSE) reaction during plus assembly *in vivo*. In this reaction, the FimG donor strand displaces the pilus assembly chaperone FimC from FimH (Fig. 1a):

\[
\text{FimC} \cdot \text{FimH} + \text{DsG} \xrightarrow{\text{DSE}} \text{FimH} \cdot \text{DsG} + \text{FimC}
\]

\[
(\text{pH} 7.0, 37^\circ C; k_{\text{DSE}} \approx 0.5 \text{M}^{-1} \text{s}^{-1})
\]

The experiments described in the following were performed with FimH from the faecal *E. coli* strain F18 (FimH\(_F18\)), which is structurally identical to the most prevalent variants in uropathogenic infection\(^25\), and FimH from the wild-type *E. coli* strain K12 (FimH\(_K12\)), which differ in three amino acids in FimH\(_K12\) (K12→F18: Val27Ala, Asn70Ser, Ser78Asn; Supplementary Fig. 1a). The isolated lectin domains (residues 1–159) of both FimH variants (FimH\(_F12\) and FimH\(_K18\)) were purified by direct expression in the *E. coli* periplasm and were purified as described\(^27\).

**Ligand-free FimH–DsG resembles FimH in the filmbrial tip.** The crystal structure of the binary complex FimH\(_F18\)–DsG was determined at atomic resolution by molecular replacement (Table 1). FimH\(_F18\)–DsG comprises the jellyroll fold FimH\(_L\) and the immunoglobulin-like FimH\(_P\) domain complemented with the FimG donor strand (Fig. 1b and Supplementary Fig. 1b). It closely resembles unliganded FimH in the filmbrial tip complex (Fig. 1c)\(^17\), with a root-mean-square deviation of Cα positions (C\(_\text{α}\) r.m.s.d.) of 1.1 Å. The individual FimH\(_L\) and FimH\(_P\) domains are even more closely resembling unliganded, filmbrial FimH (r.m.s.d. 0.45 and 0.55 Å, respectively) and undergo only a minimal hinge- bending rotation of 4° (Fig. 1c).

The DsG peptide in FimH\(_F18\)–DsG is in identical position as compared with the N-terminal FimG extension in the filmbrial tip structure; it interacts with β-strand 2 and 9 of FimH\(_P\) (Fig. 1d). All contacts in the FimH\(_L_1–FimH_P\) interdomain region (Supplementary Fig. 1c,d)\(^17\) as well as the conformation of the empty ligand-binding pocket observed in FimH in the filmbrial tip are preserved in FimH\(_F18\)–DsG. Thus, FimH\(_F18\)–DsG represents the ligand-free state of filmbrial FimH, with Associated FimH\(_L\) and FimH\(_P\) (A\(_\text{free}\) state) and is an elegant minimal system to analyse the crosstalk between ligand-binding and interdomain interactions underlying the formation of catch-bonds by FimH.
Persistence of domain association in ligand-bound FimH·DsG.

To test whether ligand-binding causes domain separation in FimH, we determined the co-crystal structure of the ternary complex of FimH\(^{\text{Fl}}\)·DsG with n-heptyl α-D-mannoside (HM), an established model ligand of FimH\(^{20}\), as well as crystal structures of the isolated FimH\(^{\text{Fl}}\) and FimH\(^{\text{Kl}}\) lectin domains in complex with HM (Table 1). FimH\(^{\text{Fl}}\)·DsG·HM adopts the same closed conformation of the ligand-binding site as previously observed in other FimHL–lectin domain complexes (Fig. 2a,b and Supplementary Fig. 2).\(^{28}\) The mannopyranose moiety of HM is coordinated by the side chains of Asp54, Gln133, Asn135 and Asp140, and the main chain of Phe1 and Asp47, and the n-heptyl aglycone of HM is sandwiched between Tyr48 and Tyr137. Compared with the \(A_{\text{free}}\) form, all loops surrounding the binding pocket close down onto the HM ligand. The most substantial conformational difference to \(A_{\text{free}}\) is observed for the clamp loop (aa. 8–16), whose tip moves almost 6 Å towards HM (Supplementary Fig. 1e,f).

Besides the closing of the ligand-binding pocket, the overall conformation of ligand-free FimH\(^{\text{Fl}}\) in \(A_{\text{free}}\) and HM-bound FimH\(^{\text{Fl}}\)·DsG is closely similar (\(C_{\alpha}\) r.m.s.d. 1.1 Å; Fig. 2a,b and Supplementary Fig. 2). Unexpectedly, the structural change in the ligand-binding site in FimH\(^{\text{Fl}}\)·DsG·HM was not transmitted to the domain interface, where the interdomain contacts and the conformations of the swing, linker and insertion loops remained intact. The lectin domain in the FimH\(^{\text{Fl}}\)·DsG·HM complex thus differs drastically from HM-bound isolated FimH\(^{\text{Fl}}\) domains with respect to the swing, linker and insertion loop conformations (Fig. 2c). On the basis of the persistence of the domain association in the ligand-bound form, this state of FimH was termed \(A_{\text{bound}}\).

To test the stability of \(A_{\text{bound}}\) state against domain separation and to exclude potential effects of selective crystallization, a molecular dynamics (MD) simulation of the \(A_{\text{bound}}\) state was conducted using the CHARMM36 force field of the
Trapping of a domain-separated state of full-length FimH. The increase in apparent affinity of FimH to its target glycans under tensile mechanical force\(^1\)^{29} has previously been linked to a separation of the FimH\(_L\) and FimH\(_P\) (ref. 17). To trap a potential domain-separated state of FimH for structural characterization in the absence of tensile force, we considered FimH variants with weakened interdomain interactions. We had shown previously the absence of tensile force, we considered FimH variants with domain-separated state of FimH for structural characterization in molecules closely resembled the state after Abound in silico removal of the HM ligand after initial equilibration, the Abound state underwent a spontaneous transition to the Afree state after ~75 ns of simulation time via an opening of the clamp loop (Supplementary Fig. 3c,d), reproducing the experimentally observed dependence of the binding-site conformation on ligand-binding. Thus, the MD simulations indicate that Abound is a stable conformational state of FimH induced by ligand-binding.

| Table 1 | Data collection and refinement statistics |
|------------------------------------------|------------------------------------------|
| Data collection                          |                                          |
| Space group                              | C 1 2 1                                  |
| Crystal dimensions                       | C 1 2 1                                  |
| a, b, c (Å)                              | 99.3, 35.5, 72.8                         |
| α, β, γ (°)                              | 90.105, 90, 90                          |
| Resolution (Å)                          | 70–1.14 (121–1.14)                      |
| R\(_{free}\)                              | 0.039 (0.760)                            |
| CC1/2                                    | 100.0 (68.0)                             |
| Compleness (%)                          | 97.6 (83.5)                              |
| Redundancy (%)                          | 3.1 (2.5)                                |
| No. reflections                         | 70.1–1.14 (87283)                        |
| No. of atoms                            | 3102                                     |
| Protein                                 | 512                                      |
| Water                                    | 26.2                                     |
| B-factors                               | 13.8                                     |
| Bond length (Å)                         | 0.009                                    |
| Bond angles (°)                         | 1.29                                     |
| Refinement                               |                                          |
| Resolution (Å)                          | 70.1–1.14 (87283)                        |
| No. reflections                         | 70.1–1.14 (87283)                        |
| No. of atoms                            | 3102                                     |
| Protein                                 | 512                                      |
| Water                                    | 26.2                                     |
| R\(_{free}\)                             | 0.039 (0.760)                            |

Values in parenthesis are for the highest resolution shell.

NAMD package (Supplementary Fig. 3a,b). The domain association remained intact over 100 ns of simulation time without substantial changes in the domain interface; fluctuations were limited to the clamp loop region close to the ligand-binding site. On in silico removal of the HM ligand after initial equilibration, the Abound state underwent a spontaneous transition to the Afree state after ~75 ns of simulation time via an opening of the clamp loop (Supplementary Fig. 3c,d), reproducing the experimentally observed dependence of the binding-site conformation on ligand-binding. Thus, the MD simulations indicate that Abound is a stable conformational state of FimH induced by ligand-binding.

To analyse the transition trajectory of the Abound to the Abound state, we removed the FimH\(_L\) domain after equilibration from the Abound state in silico for a 180-ns molecular dynamics simulation (Supplementary Fig. 3e,f). In contrast to the transition between the Abound and Afree states on ligand removal, a sharp transition to the conformation of FimH\(_L\) in the Abound state was not observed. The conformation only slowly moved towards Sbound; however, the FimH\(_L\) loops that had formed in the former interdomain interaction kept fluctuating throughout the simulation, indicating lower cooperativity and potentially a higher activation energy for the Abound to Sbound compared with the Abound to Afree transition.

A comparison of the structural dynamics in the Abound and Sbound states clearly reveals differences in the FimH\(_L\)–FimH\(_P\) interface region. The root-mean-square fluctuations of atom positions (r.m.s.f.) increase in the swing and insertion loop from a background level of ~0.7 Å in Abound to 1.5 and 2 Å in Sbound, respectively. Surprisingly, despite the virtually identical conformations of the entire ligand-binding site depicted by X-ray crystallography (Fig. 2b), the clamp loop, which exhibits the most significant conformational changes between the open and closed
conformations, exhibits strongly reduced fluctuations in $S_{\text{bound}}$, with r.m.s.f. decreasing by up to 1.5 Å (Supplementary Fig. 3g,h). This change in clamp loop dynamics provides a mechanistic link between domain association and ligand-binding in full-length FimH.

Domain association alters FimH–ligand-binding kinetics. To analyse the ligand-binding properties of FimH–DsG, we exploited the increase in intrinsic tryptophan fluorescence in the FimH–DsG complexes of ~10% on HM binding (Fig. 4a). This difference was used to measure the dissociation constant of HM binding by equilibrium titration (Fig. 4b) and the rates of HM binding and dissociation by stopped-flow fluorescence kinetics (Fig. 4c,d). The FimH–DsG constructs showed uniform binding and dissociation kinetics, consistent with the view that domain-separated states of FimH are not significantly populated in the absence of shear force. The results revealed equilibrium dissociation constants ($K_d$) of 3.6 and 9.9 μM for FimH$^{K12}$–DsG.
and FimH$^{D_{sG}}$. HM binding to FimH$^{D_{sG}}$ is extremely dynamic and was characterized by fast association rates ($k_{on}$) of $5.0 \times 10^6$ and $4.9 \times 10^6$ M$^{-1}$s$^{-1}$, respectively, and rapid dissociation reactions (Supplementary Fig. 4). The rates of HM dissociation ($k_{off}$) of 22 and 58 s$^{-1}$ for FimH$^{K_{12}}$. DsG and FimH$^{F_{18}}$. DsG translate into dissociation half-lives of only 32 and 12 ms, respectively.

In contrast to full-length FimH, isolated FimH$^{K_{12}}$ showed no change in tryptophan fluorescence on HM binding. We therefore determined the HM affinity of isolated FimH$^{L}$ indirectly by a competition experiment based on a newly designed fluorescent ligand, the fluorescein-labelled α-(1→6)-mannoside GN-FP-4 (Supplementary Fig. 5a–e and Supplementary Note 1). Displacement of GN-FP-4 from FimH$^{K_{12}}$. by increasing HM concentrations under equilibrium conditions showed that both FimH$^{K_{12}}$ and FimH$^{F_{18}}$ bind HM with 3,300-fold higher affinity compared with the respective FimH-DsG complexes ($K_d$ values 1.1 and 3.0 nM, respectively; Fig. 5a and Table 2). In an inverse competition experiment (Supplementary Fig. 5f–j), in which HM in preformed FimH$^{K_{12}}$. HM complexes was displaced by GN-FP-4, off-rates of $2.0 \times 10^{-4}$ and $3.5 \times 10^{-4}$ s$^{-1}$ were determined for FimH$^{K_{12}}$ and FimH$^{F_{18}}$, respectively, corresponding to dissociation half-lives of 58 and 33 min (Table 2). On the basis of these measured off-rates and equilibrium dissociation constants, $k_{on}$ rates of $1.8 \times 10^5$ and $1.2 \times 10^5$ M$^{-1}$s$^{-1}$ were calculated for FimH$^{K_{12}}$ and FimH$^{F_{18}}$, respectively. The on-rates for the isolated FimH$^{L}$ domains are thus 30-fold lower than those of the corresponding full-length FimH-DsG complexes.

Together, these results demonstrate that the 3,300-fold higher affinity of the isolated FimH$^{L}$ compared with full-length FimH results from a more than 100,000-fold lower ligand dissociation rate in isolated FimH$^{L}$, combined with a ligand-binding rate reduced by only 30-fold (Table 3). The 3,300-fold higher affinity for HM of FimH$^{L}$ relative to FimH-DsG translates into a free energy of 20 kJ mol$^{-1}$ for the interaction between FimH$^{L}$ and FimH$^{D_{sG}}$ in full-length FimH. This corresponds very well with the mechanical work required for domain separation, as a displacement of FimH$^{L}$ from FimH$^{P}$ by 11 Å for complete domain separation (Fig. 2a) and a force of 40 pN required to populate the domain-separated state of FimH$^{P}$ yields a value of 26.5 kJ mol$^{-1}$.

**Domain association in FimH promotes bacterial motility.** Uropathogenic *E. coli* require firm adhesion to the urinary epithelium under flow conditions to escape clearance by urine excretion. On the other hand, bacterial adhesion must be weak enough in the absence of external shear to allow flagellar motility as a prerequisite for the invasion of new tissue areas$^{22,33}$. While the role of FimH catch-bond binding for adhesion under flow conditions had clearly been demonstrated$^{22,29,34}$, the relevance of
An equimolar mixture of FimHL and GN-FP-4 (1 μM each) was incubated with different HM concentrations (10 nM–3.2 mM) for >18 h. GN-FP-4 displacement is monitored by a decrease in fluorescence polarization at 528 ± 20 nm (excitation at 485 nm). Data were fitted (solid lines) according to a mechanism in which two ligands compete for the same binding site, with fixed $K_D$ values for GN-FP-4 binding (cf. Table 2). A solution with equimolar concentrations of FimHL and HM (3 mM each, guaranteeing >95% occupancy with HM) was mixed with excess GN-FP-4 (10 μM), and the decrease in GN-FP-4 fluorescence at 520 nm as a consequence of HM dissociation and GN-FP-4 binding was recorded (Supplementary Fig. 5f–j). The obtained first-order kinetics are independent of the GN-FP-4 concentration and thus directly monitor HM dissociation.

Figure 5 | HM binding and release by the isolated FimH lectin domain FimHL. Analysis of FimH–HM interactions based on competition between HM and the synthetic fluorescent GN-FP-4 ligand. (a) HM binding to FimHL, analysed by displacement of GN-FP-4 from FimHL variants as indicated. An equimolar mixture of FimHL and GN-FP-4 (1 μM each) was incubated with different HM concentrations (10 nM–3.2 mM) for >18 h. GN-FP-4 displacement is monitored by a decrease in fluorescence polarization at 528 ± 20 nm (excitation at 485 nm). Data were fitted (solid lines) according to a mechanism in which two ligands compete for the same binding site, with fixed $K_D$ values for GN-FP-4 binding (cf. Table 2). (b) Kinetics of HM dissociation from FimHL. A solution with equimolar concentrations of FimHL and HM (3 mM each, guaranteeing >95% occupancy with HM) was mixed with excess GN-FP-4 (10 μM), and the decrease in GN-FP-4 fluorescence at 520 nm as a consequence of HM dissociation and GN-FP-4 binding was recorded (Supplementary Fig. 5f–j). The obtained first-order kinetics are independent of the GN-FP-4 concentration and thus directly monitor HM dissociation.

Table 2 | Kinetics and thermodynamics of HM binding to FimHL or FimH-ΔsG at pH 7.4 and 25°C.

| Protein | $k_{on}$ (M$^{-1}$s$^{-1}$) | $k_{off}$ (s$^{-1}$) | $k_{off}/k_{on}$ (M$^{-1}$) | $K_D$ (amplitude analysis; M$^{-1}$) | $K_D$ (equilibrium titration; M$^{-1}$) |
|---------|-----------------|----------------|----------------------|----------------------|----------------------|
| FimHL$^{K12}$ | 1.8 ± 0.6 × 10$^{10}$ | 2.0 ± 0.4 × 10$^{-4}$ | n.a | n.a | 1.1 ± 0.1 × 10$^{-1}$ |
| FimHL$^{K12}$-ΔsG | 5.0 ± 0.1 × 10$^{10}$ | 2.2 ± 0.1 × 10$^{-3}$ | 4.3 ± 0.1 × 10$^{-6}$ | 4.2 ± 0.3 × 10$^{-6}$ | 3.6 ± 0.3 × 10$^{-6}$ |
| FimHF$^{18}$ | 1.2 ± 0.4 × 10$^{10}$ | 3.5 ± 0.8 × 10$^{-4}$ | n.a | n.a | 3.0 ± 0.2 × 10$^{-9}$ |
| FimHF$^{18}$-ΔsG | 4.9 ± 0.1 × 10$^{10}$ | 5.8 ± 0.1 × 10$^{-3}$ | 1.2 ± 0.4 × 10$^{-5}$ | 1.2 ± 0.1 × 10$^{-5}$ | 9.9 ± 1.5 × 10$^{-6}$ |

HM, α-heptyl α-o-mannoside. The rate constants $k_{on}$ and $k_{off}$ were determined from experiments as shown in Figs 4 and 5. The $K_D$ values for the complex formation between HM constructs and the different FimH constructs were determined (a) from the ratio of rate constants ($k_{off}/k_{on}$), (b) from the analysis of the amplitudes as in Fig. 4d and (c) from equilibrium titration as in Fig. 4b. (d) $K_D$ values were calculated with $k_{on}$ and $k_{off}$. (e) Values of $K_D$ were obtained from competition equilibria with the fluorescent mannoside GN-FP-4 (Fig. 5a) and the following $K_D$ values of GN-FP-4 binding determined in Supplementary Fig. 5: FimHF$^{18}$: $K_D = 7.0 ± 0.1 × 10^{-10}$ M; FimHF$^{18}$: $K_D = 1.8 ± 0.2 × 10^{-10}$ M.

Table 3 | Comparison of HM binding by variants of FimHL versus FimH-ΔsG.

| FimH variant | $k_{on}$ (FimH-ΔsG)/$k_{on}$ (FimHL) | $k_{off}$ (FimH-ΔsG)/$k_{off}$ (FimHL) | $K_D$ (FimH-ΔsG)/$K_D$ (FimHL) |
|--------------|-------------------------------|-------------------------------|-------------------------------|
| K12 | 28 | 110,000 | 3,300 |
| F18 | 41 | 170,000 | 3,300 |

HM, α-heptyl α-o-mannoside.

The fraction of cells permanently stayed attached after adhesion to 1M-BSA until binding/detachment, but with fivefold longer adhesion (35.2 s; Supplementary Fig. 7d). Remarkably, the fraction of cells that permanently stayed attached after adhesion to 1M-BSA until
Figure 6 | Cell-tracking analysis of bacterial motility on mannosylated surfaces. *E. coli* cells piliated with FimH*<sup>F18</sup>* or the FimH*<sup>F18</sup>-Ala188Asp variants were tracked under static conditions in the absence of shear force. (a) The fraction of bacteria attached to mannose-coated (1M-BSA) or BSA-coated surface (negative control) at the beginning of the time-lapse movies (white bars) and after 5 min (black bars) are given. Bacterial motility on 1M-BSA was analysed in the absence and presence of HM. The delay between application of bacteria and movie recording was ~1 min. (b) Fraction of tracked cells that were pre-attached (yellow; speed < 0.5 μm s<sup>-1</sup>), permanently attach (red), were mobile (white), transiently attach (green) or permanently detach (blue) during the entire observation time (5 min). Right: schematic depiction of the observed cell behaviour. FimH*<sup>F18</sup>-piliated *E. coli* show almost exclusively transient attachment events on 1M-BSA. FimH*<sup>F18</sup>-Ala188Asp-piliated *E. coli* show less transient attachment but enhanced permanent attachment to 1M-BSA. Transient and permanent attachment to 1M-BSA is significantly reduced in the presence of HM. For each experiment five to seven independent replicates were analysed.

![Diagram](image1.png)

**Figure 7 | Catch-bond mechanism of FimH-mediated cell adhesion.** (a) In the absence of tensile mechanical force, formation of the FimH-Uroplakin 1a (UPIa) complex comprises the highly dynamic transition of the A<sub>free</sub> to the A<sub>bound</sub> state. The reaction likely proceeds via a transient encounter complex (indicated in square brackets). The reaction of the encounter complex to A<sub>bound</sub> is not rate-limiting and must have a half-life of less than 1 ms. Dissociation of the receptor from the FimH lectin domain in the A<sub>bound</sub> state is promoted via dynamic allostery by the pilin domain that acts as a negative allosteric regulator. The reaction from A<sub>bound</sub> to the encounter complex corresponds to k<sub>off</sub>. Fast binding and release of UPIa by FimH enables bacterial motility on the bladder epithelium. (b) Shear force increases the population of the S<sub>bound</sub> state of FimH, in which the pilin and lectin domains are separated. The dissociation of S<sub>bound</sub> under shear force is slowed down up to 100,000-fold compared with A<sub>bound</sub>. The indicated rate constants and half-lives correspond to the interaction between FimH*<sup>F18</sup>* and the model ligand HM. Rate limiting reactions are indicated by solid arrows, and fast, non-limiting reactions by dashed arrows.

The end of the observation period was much larger for FimH*<sup>F18</sup>-Ala188Asp (11.5%) than for FimH*<sup>F18</sup> (0.6%; red in Fig. 6b). Those permanently attached cells escape kinetic analysis; thus, the true average attachment time for FimH*<sup>F18</sup>-Ala188Asp must be considerably larger than 35.2 s. Permanent attachment is also the main cause of the increased fraction of attached cells for FimH*<sup>F18</sup>-Ala188Asp-piliated bacteria (Fig. 6a).

Altogether, cell-tracking analysis revealed that enforced domain separation in the FimH*<sup>F18</sup>-Ala188Asp variant resulted in reduced detachment rates and a larger proportion of permanently attached cells. These results directly demonstrate, at the cellular level, the importance of fast, spontaneous ligand dissociation catalysed by interdomain allostery in FimH–ligand complexes for bacterial motility in the absence of tensile mechanical forces.

**Discussion**

The characterization of full-length FimH had so far been restricted to the analysis of the adhesive properties of piliated *E. coli* cells and binding studies with the purified type 1 pilus tip fibrillum. With the FimH·DsG complex, we have now established a model system for quantitative studies of the interaction of FimH with carbohydrate ligands. Soluble FimH·DsG efficiently mimicks FimH in the context of the assembled tip fibrillum, is readily available in milligram quantities and permits the determination of ligand-binding and release kinetics in solution.
Using FimH-DsG, we obtained high-resolution snapshots of three functionally relevant states of FimH (Fig. 7). In the absence of ligands, FimH adopts the A\text{free} state with associated FimH_L and FimH_P and an open conformation of the ligand-binding site, which is responsible for the 30-fold faster ligand-binding of full-length FimH as compared with the isolated FimH_L domain. Ligand-binding in the absence of shear force induces the A\text{bound} state with a closed binding site. In contrast to earlier hypotheses\cite{17}, the transition from A\text{free} to A\text{bound} is restricted to the ligand-binding site, while all interactions between FimH_L and FimH_P observed in the A\text{free} state remain preserved in A\text{bound}.

The A\text{free}→A\text{bound} transition most likely follows an induced fit mechanism, in which the formation of an encounter complex between FimH-DsG and HM is rate-limiting and followed by a fast, unimolecular rearrangement to the A\text{bound} state, in agreement with the observation that binding of the model ligand HM remained rate-limiting for the formation of A\text{bound} even at the highest HM concentrations used. Stopped-flow-binding kinetics indicate that the lifetime of the proposed encounter complex before A\text{bound} formation is below 1 ms (Fig. 4c). Under tensile mechanical force applied to the FimH-ligand complex, mimicked here by the destabilized variant FimH\text{L} in the S\text{bound} state, in FimH_L and FimH_P no longer interact specifically and are only connected via the linker segment comprising FimH residues 154–160. In this S\text{bound} state, FimH_L adopts a conformation closely resembling isolated FimH_P with bound ligand.

Notably, ligand dissociation from FimH-DsG is 100,000-fold faster than that from the isolated FimH_L domain. This is striking because the respective crystal structures revealed indistinguishable ligand interactions and binding-site conformations in the FimH-DsG-HM and FimH_L-HM complexes (Fig. 2b). MD simulations identified a considerable increase in the conformational dynamics of the FimH_L–ligand-enclosing clamp loop in the A\text{bound} state as the most likely cause of the dramatic increase in k_{diss} in the FimH-DsG-HM complex. The altered dynamics in FimH_L in the A\text{bound} state are the result of the presence of FimH_P, which can be described as a negative allosteric regulator\cite{36,37}. The allosteric communication from the FimH_L–FimH_P interface to the ligand-binding site reaches over 40 Å, and is mediated via changes in protein dynamics rather than in static structure, in line with a general model of dynamic allostery\cite{39,40}. Our data demonstrate that the interdomain interactions in FimH (i) maintain the open conformation of the binding pocket and guarantee rapid ligand-binding and (ii) intramolecularly catalyse ligand dissociation by more than 100,000-fold. Rapid ligand-binding and short lifetimes of the FimH ligand complex allow for rapid dissociation of individual pili from their ligands in the absence of shear force. Our biophysical data demonstrate that this mechanism is conserved between the K12 and the F18 strains.

Different mechanistic models, such as the two-pathway\cite{41}, the deformation\cite{42} and the sliding re-binding model\cite{43}, have been developed to describe catch-bond interactions, often based on powerful single-molecule atomic force measurements. These models included the principle of allosteric control of ligand-binding affinity\cite{26,31}, which was clearly confirmed in our present study. However, these conceptual models did not reveal the underlying atomic-scale mechanisms in different catch-bond systems. For most catch-bond systems, including the cadherin–catenin binding to actin filaments\cite{33}, integrin epithelial cell adhesion\cite{15,46} and TCR–MHC interactions\cite{6,47}, structural information is, if at all, available only for one state or from computer simulations. One exception is the selectins, which employ catch-bond binding for leukocyte recruitment. Selectins are multidomain cell surface receptors, which consist of a lectin domain for complex carbohydrate binding, linked via an epidermal growth factor (EGF)-like domain to a variable number of short consensus repeat domains and a transmembrane-anchoring helix. Selectins exist in two conformations, a bent and an extended one, which differ in the angle between their lectin and EGF-like domain. Ligand-binding and conformational changes in the ligand-binding site are directly linked via a complex allostERIC coupling mechanism to the adoption of the extended conformation\cite{48,49}. Tensile mechanical force under flow conditions acts along the axis of the ligand-binding site and the LeC-EGF interface resulting in a stabilization of the extended conformation and thus increased ligand complex lifetimes\cite{24}. Moreover, in FimH, catch-bond behaviour is mediated by the interplay of a lectin and an anchoring domain that does not interact with the ligand. Ligand-binding by FimH in the absence of shear force results in a closing of the ligand-binding site, but, in contrast to selectins, is not directly linked to altered interdomain interactions. Here mechanical force promotes domain separation and completely releases FimH_L from FimH_P, which acts as an activator of ligand release via dynamic allostery. Remarkably, selectins and the fimbrial adhesin FimH thus employ entirely different mechanisms for establishing catch-bond behaviour by crosstalk between the lectin and an anchoring domain that provides tethering to a shaft. In both systems, the selectins and fimbrial adhesion, the shaft structures linking the terminal lectin/coupling domains to the cell surface, may contribute to the overall catch-bond behaviour, either via directly influencing coupling domain behaviour or via their general elastic properties\cite{50,51}.

The cell-tracking experiments indicate the importance of rapid ligand release from the high-mannose-type glycoprotein receptor uroplakin 1a in the lower urinary tract\cite{42} for flagellar motility of piliated bacteria, and hence their ability to colonize new tissue areas under certain conditions during infection\cite{12,29,53}. This provides a plausible explanation for the fact that low-affinity FimH variants were preserved in numerous uropathogenic E. coli strains. Binding of terminal mannoses with low affinity in the absence of shear force may also play a role in preventing the clearance of uropathogenic E. coli from the urinary tract by competitive binding to the Tamm–Horsfall protein in the urine\cite{54}.

In turn, populating the S\text{bound} state with an extremely low dissociation rate ensures tight bacterial adhesion under the mechanical forces of urine excretion. FimH is a promising target for anti-adhesive therapy of UTI because FimH antagonists, in contrast to antibiotics, are not exerting selection pressure towards resistance formation\cite{18,55,56}. Previous ligand-binding studies on the isolated FimH_L domain mimic the domain-separated S\text{bound} state of FimH. This state is characterized by extremely low off-rates and is promoted in vivo only after ligand-binding and the onset of flow conditions. Our kinetic data on ligand dissociation from full-length FimH demonstrate that rapid, competitive displacement of FimH from its carbohydrate ligands by FimH antagonists is well possible in the absence of shear force. Thus, full-length FimH (for example, in the form of the FimH-DsG complex established in this study) instead of the isolated FimH_L domain is the relevant target for the development of anti-adhesive drugs. Importantly, the concept of the FimH-DsG model system can now be expanded to other related adhesive pilus adhesins. In combination with the novel fluorescent GN-FP-4 ligand, this model system paves the way for efficient screening for anti-adhesive drug candidates.

**Methods**

**Materials.** The synthetic DsG (sequence: ADVTITVNGKVVAKR) and DsF peptide (sequence: ADSTTIRGTVRDN; > 95% purity) were purchased from JPT (Germany). Guanidinium chloride (‘AA-Grade’ for spectroscopy) was
obtained from NIGU Chemie (Germany). Standard chemical of highest purity available was obtained Sigma, Merck or ApplChem. If not mentioned otherwise, chromatography media for protein purification were purchased from GE Healthcare (UK). Oligonucleotides were from Microsynth (Switzerland).

Construction of expression plasmids. Expression plasmids for the periplasmic production of the E. coli FimH lectin domain (FimH<sub>ND</sub>) and for the periplasmic co-expression of full-length FimF<sub>ND</sub> with FimC were based on the expression plasmids pMMH<sub>ND</sub> and pMMH-fimC-ATG, respectively, for the analogous proteins from E. coli K12 (ref. 27). Six silent mutations replacing rare codons were introduced to the E. coli FimH<sub>ND</sub> gene (fimH<sub>ND</sub>) contained in the plasmid pGB2-24 (ref. 57) with the QuickChange mutagenesis kit (Agilent Technologies, Switzerland) to improve periplasmic expression. The coding sequence of the modified fimH<sub>ND</sub> gene was amplified by PCR using the primers 5′-GAAAGGAGGAG TGGTTACATG TAAAACCAATTACG-3′ and cloned into pMMH<sub>ND</sub>-fimH<sub>ND</sub>277 and into the HindIII sites of plasmids pBluescript II SK- and pBluescript II SK+ and used for transformation of E. coli DH5α. Protein production in the resulting plasmid pMMH<sub>ND</sub>277 is under control of the trc–promoter/ lac operator.

Protein production and purification. For purification of the complexes FimC–FimH<sub>ND</sub>277 and FimC–FimH<sub>ND</sub>F18, E. coli HM125 harbouring the corresponding co-expression plasmid was grown at 30°C in 2YT medium containing ampicillin (100 μg·ml<sup>−1</sup>) at an OD<sub>600</sub> of 1.5, isopropyl-β-D-thiogalactoside (IPTG) was added to a final concentration of 1 mM. The cells were further grown for 12–18 h at 4°C. After centrifugation, suspended in cold 50 mM Tris·HCl pH 7.5, 150 mM NaCl, 5 mM EDTA, 1 mM monoxymonosulphate B sulphate (18 mM·l<sup>−1</sup> of culture) and stirred at 4°C for 1.5 h. After centrifugation, the supernatant (periplasmic extract) was dialysed against 20 mM MOPS–NaOH pH 8.0 and applied to a QAE-52 (Whatman, Maidstone, UK) column equilibrated with the same buffer. The flow-through containing the respective FimC–FimH<sub>ND</sub> complex was dialysed against 20 mM MOPS–NaOH pH 8.0, loaded onto a Resource S (6 ml) column equilibrated with the same buffer and the complexes were eluted with a linear NaCl gradient (0–100 mM). The protein was eluted with a linear NaCl gradient (0–600 mM), and loaded onto a Superdex 75 (HiLoad 26/60) column equilibrated with 20 mM NaH<sub>2</sub>PO<sub>4</sub>–NaOH QA52 (Whatman, Maidstone, UK) column equilibrated with the same buffer. The complexes were eluted with a linear NaCl gradient (0–600 mM). Fractions containing pure FimHL were pooled, dialysed against 20 mM acetic acid–NaOH pH 4.5 containing pure FimHL were pooled, dialysed against water and stored at -80°C. The FimHL<sub>DsG</sub> complex and excess DsG was dialysed against 20 mM acetic acid–NaOH pH 4.5. The protein was eluted with a linear NaCl gradient (0–400 mM). Fractions containing FimC were pooled, dialysed against 20 mM acetic acid and evaporated. Chromatography on silica gel (CH<sub>2</sub>Cl<sub>2</sub>/MeOH) yielded 23 mg (0.042 mmol, 68%) of tert-butyl (3-chloro-4-((3′-d-mannopyranosyloxy)-biphenyl-4-yl)carboxamido)ethyl carbamate. This product was dissolved in CH<sub>3</sub>Cl<sub>2</sub> (3 ml) and trifluoroacetic acid (TFA, 1 ml) was added. The solid dissolved during addition of TFA. The mixture was evaporated at room temperature the reaction was complete. The mixture was evaporated and excess TFA was removed in high vacuum. The intermediate N-(2-aminoethyl)-3′-chloro-4′-((3′-d-mannopyranosyloxy)-biphenyl-4-yl)carboxamide TFA salt (23 mg, 0.042 mmol, quant.) was used without purification in the next step. After addition of dry DMF (200 μl), triethylamine (12.8 mg, 0.127 mmol) was added. The mixture was cooled to 0°C, triethylamine (14.8 mg, 0.038 mmol) was added and the mixture was stirred for 3 h in the dark. After the addition of water, DMF was removed azeotropically, the residue dissolved in MeOH/10% acetic acid and evaporated. Chromatography on silica gel (CH<sub>3</sub>Cl<sub>2</sub>/MeOH) yielded compound 2, contaminated with triethylammonium acetate. Therefore, after complete dissolution in MeOH, 0.5 N HCl in MeOH was added, the mixture evaporated and chromatographed on silica gel to yield pure compound GP-F4-1 (3′-Chloro-N-2-(3′,3′-diidroxy-3-oxo-3-hydroxypropyl)-[3′-α,α′-bifluoro]-trithiocarbamide-ethyl)ethylformamide (compound 1; calculated mass: 10,291.57; measured mass: 10,291.82 (m/z), 792.0 (d, J = 8.3, 2H, Ar–H), 770.7 (d, J = 5.0, 13.1, 2H, Ar–H), 7.64 (d, J = 8.3, 2H, Ar–H), 7.54 (dd, J = 2.2, 8.6, 1H, Ar–H), 7.46 (d, J = 8.7, 1H, Ar–H), 7.09 (d, J = 8.2, 1H, Ar–H), 6.74 (d, J = 1.4, 2H, Ar–H), 6.65 (d, J = 8.4, 2H, Ar–H), 5.63 (d, J = 1.3, 2H, H-1), 4.15 (d, J = 1.8, 3.1 Hz, H-2), 4.03 (d, J = 3.4, 9.5 Hz, H-3), 3.94 (s, 2H, CH<sub>2</sub>), 3.86-3.64 (m, 6H, H-4, H-5, H-6, H-7); 13C NMR (126.8 Hz, CD<sub>3</sub>OD); δ = 135.21, 134.83, 134.81, 126.99, 126.19, 127.76, 127.70, 125.37, 118.64, 103.62 (Ar–C), 100.75 (C–1), 76.00 (C–5), 72.41 (C–3), 71.86 (C–2), 62.84 (C–4), 62.69 (C–6), 40.76 (CH<sub>3</sub>); ESI-MS: m/z: Calculated for C<sub>26</sub>H<sub>35</sub>N<sub>5</sub>O<sub>7</sub> [M + H]<sup>+</sup>: 842.2; found: 842.2.

Fluorescence spectroscopy. Fluorescence emission spectra of FimH variants were recorded between 300 and 450 nm (excitation at 280 nm) at 25°C in 1.0 × 0.4 cm quartz cuvettes on a QM 7/2003 spectrofluorimeter (PTI, USA) equipped with a motorized stage. Protein concentration in the buffer was 1–2 μM and rinsed with 0.5 M NaH<sub>2</sub>PO<sub>4</sub>–NaOH pH 7.4. Fluorescence spectra of GP-F4-1 (0.055 μM) = 54,900 M<sup>−1</sup> cm<sup>−1</sup>) was recorded between 500 and 650 nm (excitation at 497 nm) in the same buffer.

Kinetics of HM binding to FimH–DsG. The rate constants of binding (k<sub>b</sub>) and dissociation (k<sub>d</sub>) for the complex between FimH–DsG and HM were measured at 25°C in 20 mM MOPS–NaOH pH 7.4 in a 250 μl stopped-flow instrument (Applied Photophysics, UK). A constant FimH–DsG concentration of 1 or 2 μM was used. FimH–DsG was mixed with different concentrations of HM (1–100 μM), and binding was monitored by the increase in fluorescence above 320 nm (excitation at 280 nm). The fluorescence traces were globally fitted with Dynafit<sup>59</sup> according to a two-binding-site and first-order dissociation reaction. As an additional control, the fluorescence amplitudes of the individual reactions were plotted against the HM concentration and fitted according to equation (2). The deduced dissociation constants reproduce the K<sub>d</sub> values obtained with equilibrium titration within experimental error.

Equilibrium titration of FimH–DsG with HM. The binding equilibrium between FimH–DsG and HM were followed at 25°C in 20 mM MOPS–NaOH pH 7.4 on a QM 7/2003 spectrofluorimeter (PTI) by the increase in fluorescence above 320 nm on HM binding (excitation at 280 nm). Measurements were performed with a stirred 1 × 0.4 cm quartz cuvette. The concentration of FimH–DsG was kept constant at 2 μM while the concentration of HM was varied between 0.1 and 100 μM. The samples were equilibrated overnight, and their fluorescence intensities were recorded for 30 s and averaged. The fluorescence intensities were plotted against total HM
concentration and fitted according to equation (2)

\[
F = \left( F_0 - F_\infty \right) \left[ \frac{[\text{p}]+[\text{i}]}{K_d} + K_d \sqrt{\frac{[\text{p}]+[\text{i}]}{K_d}} \right]^2 - \frac{[\text{p}]+[\text{i}]}{K_d} + F_\infty
\]

where \( F \) is the measured fluorescence signal, \( F_0 \) is the fluorescence signal in the absence of ligand, \( F_\infty \) is the fluorescence signal at full saturation with ligand, \( K_d \) is the dissociation constant, \([\text{p}]_0\) is the total concentration of FimH-DsG and \([\text{i}]_0\) is the total concentration of HM.

**Equilibrium titration of FimH with GN-FP-4.** The binding equilibrium between FimH, and GN-FP-4 at 25°C in 20 mM MOPS–NaOH pH 7.4 was measured indirectly by binding of excess GN-FP-4 to FimH, after dissociation of HM, recorded with the decrease in GN-FP-4 fluorescence at 520 nm (excitation at 497 nm). Measurements were performed with a stirred 1 × 0.4 cm quartz cuvette. The concentration of GN-FP-4 was kept constant at 0.2 M Na Malonate, 20% PEG3350 within 2 months at 4°C.

**Displacement of HM from the FimH by GN-FP-4.** The rate constant of dissociation \((k_{\text{off}})\) for HM from FimH at 25°C in 20 mM MOPS–NaOH pH 7.4 was determined by the competition between HM and GN-FP-4 for binding to FimH. A mixture of FimH and GN-FP-4 (1 μm each) was incubated with different concentrations of HM for 10–3.2 mM, and GN-FP-4 fluorescence was recorded every 10 min for 10 s, averaged and the data fitted according to first-order kinetics. The obtained rate constants were independent of GN-FP-4 concentration and thus identical to the dissociation rate of HM from FimH.

**Determination of the FimH–HM dissociation constant.** The affinity of FimH for HM at 25°C in 20 mM MOPS–NaOH pH 7.4 was determined by the competition between HM and GN-FP-4 for binding to FimH. A mixture of FimH and GN-FP-4 (1 μm each) was incubated with different concentrations of HM (10–3.2 mM) and incubated for at least 18 h. The displacement of GN-FP-4 by HM was recorded on the decrease in the fluorescence polarization at 528 ± 2 nm (excitation at 485 ± 20 nm) on a microplate reader (Biotek, USA), using flat black-bottom 96-well microtiter plates (Greiner, Austria). The fluorescence polarization data were fitted with DynaFit 5.9a according to an equilibrium competition mechanism, with the total concentrations of FimH, GN-FP-4 and HM (variable) and the respective \( K_d \) of GN-FP-4 (Table 2) as input, and \( K_d \) of HM and the fluorescence polarization at zero and infinite HM concentration as open parameters.

**Crystallization of FimH variants.** All crystallization experiments were performed at 4°C with the sitting drop vapour diffusion method. For crystallization, FimH\(^{18}\), DsG and FimF\(^{18,2}\) DsG (0.1–0.2 μl, 15 mg ml\(^{-1}\) in H2O) was mixed with 0.1 M BisTris-HCl pH 5.5 at 4°C. Crystallization of FimH\(^{18}\), DsG and FimF\(^{18,2}\) DsG grew within 4–6 weeks and the concentration of FimH\(^{18}\) was varied between 0 and 10 μM. The samples were equilibrated overnight, and their fluorescence intensities at 520 nm were recorded for 30 s and averaged. The experimental data were fitted according to equation (2).

**Cell tracking on mosaically-BSA-coated surfaces.** The E. coli KB18 strain was kindly provided by Professor Evgeni Sokurenko and served as host for the generation of recombinant strains. KB18 contains the pPKL114 plasmid, which encodes the whole fim operon with a translational stop linker upstream of the fimH gene. KB18 was co-transformed with the pb2G-24 plasmid, which was isolated from the ELT115 strain and encodes fimBP (kindly provided by Professor Evgeni Sokurenko). Single-nucleotide point mutations were introduced in fimBP using overlap extension PCR following standard molecular techniques to obtain fimFP\(^{18}\) and fimFP\(^{18}\)-Ala188Asp. The PCR products were cloned into pb2G-24 by the Apal and Spil sites, and KB18 was transformed with the resulting plasmid.

**Cell culture dishes (35 mm, Corning Inc., Corning, NY) were incubated with 50 μl of 50 μg ml\(^{-1}\) 1 M-BSA in 0.2 M bicarbonate buffer for 75 min at 37°C.** The dishes were then washed three times and quenched with 0.1% PBS–BSA (ref. 63) until late log phase (OD\(_{600}\) of 1.0–1.2) and diluted to an OD\(_{600}\) of 0.01 before acquisition.

Cell tracking was carried out at room temperature under static conditions. A bacterial suspension of 50 μl in the late logarithmic growth phase was placed onto the cell culture dishes (diluted to OD\(_{600}\) of 0.01), and a cover slide was placed on top. The delay between sample placement and start of the movie acquisition was about 1 min. Time-lapse movies were recorded with a ×20 phase contrast microscope using a CMOS digital camera (The Imaging Source, Dusseldorf, Germany) mounted on a Nikon Ti Eclipse inverted microscope and using the NIS Elements Basic Research software (Nikon, Zurich, Switzerland). Phase contrast images in an ~3-μm-thick surface layer were taken at four to five frames per second over 5 min. The dead time of movie acquisition was ~1 min. The resulting images were segmented using a projection of the average intensities over all frames to remove the background and by subsequent thresholding using the Maximum Entropy method in Fiji to obtain binary images (examples shown in Supplementary Movies 1 and 2). The segmented images were imported into Imaris (Bitplane, Zurich, Switzerland) and tracked through the autoregressive track algorithm. A time filter was applied to exclude all tracks with a length below 15 s. Tracking times for 15 s were reviewed individually and edited manually, if necessary. Five to seven independent movies were recorded for each experimental set-up: FimFP\(^{18}\) or FimFP\(^{18}\)-Ala188Asp binding to 1 M-BSA in the absence (1.815 ± 1.283 individual tracks, respectively) and in the presence of 200 μM HM (1.175 ± 1.071 individual tracks, respectively) were analysed respectively. For E. coli plated with FimH\(^{18}\) or FimH\(^{18}\)-Ala188Asp binding to BSA in the absence (1.815 ± 1.283 individual tracks, respectively) and in the presence of 200 μM HM (1.175 ± 1.071 individual tracks, respectively) were analysed respectively. For E. coli plated with FimFP\(^{18}\) or FimFP\(^{18}\)-Ala188Asp binding to BSA 1.314 ± 1.065 individual tracks, respectively, were analysed. Bacteria with a speed of <0.5 μm s\(^{-1}\) were classified as attached, all other bacteria were classified as mobile. In addition, a limitation in the spatial and temporal resolution of movie acquisition, we did not further subdivide bacterial swimming into motility behaviours as ‘rolling’ and ‘progression’ and so on. The individual cell tracks were classified into four classes: no motility change during observation (pre-attached or mobile), transient attachment and permanent attachment, and permanent attachment. For FimFP\(^{18}\) on 1 M-BSA surfaces, 13.9% (251 out of 1,815 tracks) of all tracks showed a single track, respectively, were analysed. Bacteria with a speed of <0.5 μm s\(^{-1}\) were classified as attached, all other bacteria were classified as mobile.
bacteria that underwent a first transient adhesion attached and detached from the surface a second time. For these cells, the average time between detachment and re-attachment was only 13.5 s (Supplementary Fig. 7e), suggesting that re-binding may be favoured by proximity to the surface as compared with the initial attachment. The mean velocity on 1M-BSA, as compared with BSA-coated surfaces, was reduced for both FimH\(^{18}\)-piliated (4.2 and 7.4 \(\mu m/s\), respectively) and FimH\(^{18}\)-Ala188Asp-piliated bacteria (3.5 and 8.1 \(\mu m/s\), respectively; Supplementary Fig. 7a,b). This reduction of the mean velocity originates from two different phenomena: in FimH\(^{18}\)-piliated cells it is caused by a change from fast swimming to a slower mode of motion (Supplementary Fig. 7b; Supplementary Movie 1), which is consistent with bacterial surface rolling due to weak, short-lived mannoside interactions.\(^{29,25}\) In contrast, for FimH\(^{18}\)-Ala188Asp-piliated bacteria, the reduction of the mean velocity results from an increase in the fraction of adherent cells on 1M-BSA compared with BSA (see main text). In the presence of 200 \(\mu\)M HM, the mean velocity on 1M-BSA is increased for both FimH\(^{18}\)-piliated (6.5 \(\mu m/s\)) and FimH\(^{18}\)-Ala188Asp-piliated bacteria (5.9 \(\mu m/s\); Supplementary Fig. 7a) and transient and permanent attachment is reduced by 75% and 85%, respectively (Fig. 6 and Supplementary Fig. 7c).

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Author contributions

M.M.S., R.P.J., R.G. and T.M. conceived the study and experimental approach; M.M.S. and J.E. cloned, purified and crystallized proteins and carried out kinetic experiments; R.P.J. crystallized proteins, collected X-ray diffraction data and determined crystal structures; G.N. and B.E. synthesized GN-FP-4. Se.B. and Si.B. performed molecular dynamics simulations. D.E. and R.P.J. performed cell-tracking experiments; and M.M.S., R.P.J., J.E., B.E., R.G. and T.M. wrote the paper with input from all authors.

Additional information

Accession codes: The atomic coordinates and structure factors have been deposited in the Protein Data Bank under the accession codes 4XO8, 4XO9, 4XOA, 4XOB, 4XOC, 4XOD, 4XOE.

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