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DOI: 10.1038/nchembio.1019

Posted at the Zurich Open Repository and Archive, University of Zurich
ZORA URL: http://doi.org/10.5167/uzh-65054

Originally published at:
Crespo, Maria D; Puorger, Chasper; Schärer, Martin A; Eidam, Oliv; Grütter, Markus G; Capitani, Guido; Glockshuber, Rudi (2012). Quality control of disulfide bond formation in pilus subunits by the chaperone FimC. Nature Chemical Biology, 8(8):707-713. DOI: 10.1038/nchembio.1019
Quality control of disulfide bond formation in pilus subunits by the chaperone FimC

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Type 1 pili from uropathogenic *Escherichia coli* are filamentous, noncovalent protein complexes mediating bacterial adhesion to the host tissue. All structural pilus subunits are homologous proteins sharing an invariant disulfide bridge. Here we show that disulfide bond formation in the unfolded subunits, catalyzed by the periplasmic oxidoreductase DsbA, is required for subunit recognition by the assembly chaperone FimC and for FimC-catalyzed subunit folding. FimC thus guarantees quantitative disulfide bond formation in each of the up to 3,000 subunits of the pilus. The X-ray structure of the complex between FimC and the major pilus subunit FimA and the kinetics of FimC-catalyzed FimA folding indicate that FimC accelerates folding of pilus subunits by lowering their topological complexity. The kinetic data, together with the measured in vivo concentrations of DsbA and FimC, predict an in vivo half-life of 2 s for oxidative folding of FimA in the periplasm.

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Type 1 pili from uropathogenic *Escherichia coli* strains are filamentous, highly oligomeric protein complexes anchored to the outer bacterial membrane and are responsible for the attachment of the bacteria to the host tissue. All structural pilus subunits are homologous proteins sharing an invariant disulfide bridge. Here we show that disulfide bond formation in the unfolded subunits, catalyzed by the periplasmic oxidoreductase DsbA, is required for subunit recognition by the assembly chaperone FimC and for FimC-catalyzed subunit folding. FimC thus guarantees quantitative disulfide bond formation in each of the up to 3,000 subunits of the pilus. The X-ray structure of the complex between FimC and the major pilus subunit FimA and the kinetics of FimC-catalyzed FimA folding indicate that FimC accelerates folding of pilus subunits by lowering their topological complexity. The kinetic data, together with the measured in vivo concentrations of DsbA and FimC, predict an in vivo half-life of 2 s for oxidative folding of FimA in the periplasm.

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Results

FimC only interacts with disulfide-intact unfolded FimA

FimC is a monomeric two-domain protein (22.7 kDa) that interacts with pilus subunits mainly via its N-terminal domain¹⁵⁻²⁰ (the structure of the FimC–FimA complex is described in detail below). The only two tryptophan residues (Trp36 and Trp84) of FimC are located in its N-terminal domain, close to the subunit-binding site. As FimA lacks tryptophan residues, we first tested whether binding of FimA can be detected via a tryptophan fluorescence change in FimC. Figure 2a shows that addition of a two-fold excess of FimA$_{ox}$ to FimC under native conditions resulted in a ~25%
Figure 1 | Schematic of type 1 pilus assembly by the chaperone-usher pathway. (a) The periplasmic chaperone FimC forms complexes with the newly translocated pilus subunits (FimA, FimG, FimF and FimH) in the periplasm that diffuse to the assembly platform (usher) FimD. The usher then catalyzes pilus subunit assembly and mediates translocation of folded subunits through the outer membrane2,10. Subunits are only assembly competent when bound to FimC2,10. OM, outer membrane; IM, inner membrane. (b) Topology diagram of the main structural pilus subunit FimA. The location of the single, conserved disulfide bond that connects the N terminus of the first A strand with the C terminus of the second B strand is depicted in yellow with the corresponding residue numbers. The polypeptide segment of FimC that complements the FimA fold in FimC–FimA complex is indicated in red.

Figure 2 | Complex formation between FimA red and FimC at pH 7.0 and 25 °C. (a) Tryptophan fluorescence spectra of 2 µM FimC, 4 µM FimA red, 4 µM FimA red, a mixture of 2 µM FimC and 4 µM FimA red, and a mixture of 2 µM FimC and 4 µM FimA red (excitation at 280 nm). Dashed black line indicates the sum of the spectra of 2 µM FimC and 4 µM FimA red. (b) Stopped-flow fluorescence kinetics of the refolding of 3 µM FimA red (solid black trace) and 3 µM FimA red (dotted black trace) in the presence of 1 equiv. FimC (3 µM FimC). The solid red line represents a fit according to a second-order reaction (equation (1)). (c) Analytical ion exchange chromatography of complex formation. FimA red or FimA red was mixed with a two-fold excess of FimC (5 µM) and free FimA were separated by cation exchange chromatography at 4 °C. The identity of the different peaks was verified by SDS-PAGE (Supplementary Fig. 2). Addition of DsbA red to the mixture of FimC and FimA red rescued formation of the FimC–FimA complex. Runs with isolated FimA red and isolated FimC are also shown.

Oxidation of FimA red by DsbA rescues binding to FimC

We next investigated the ability of FimC to selectively bind FimA red using analytical cation-exchange chromatography. This allowed direct detection of the native FimC–FimA complex and the rescuing of unfolded FimA red for binding to FimC by oxidation with DsbA. Figure 2c shows that FimA red formed a complex with FimC within the dead time of the experiment (about 1 min) after rapid dilution of FimA red into native buffer containing a two-fold excess of FimC. Formation of the FimC–FimA complex was detected via an additional peak and a decrease in the FimC peak intensity (Fig. 2c and Supplementary Fig. 2). When the same experiment was performed with FimA red instead of FimA red, no FimC–FimA complex was formed even after incubation for 24 h, and FimA was detected in the flow-through (Fig. 2c). However, when an excess of oxidized DsbA (DsbA red) relative to FimA red was added to the mixture of FimA red and FimC before application to the column, the FimC–FimA complex was fully recovered (Fig. 2c). FimA red is thus unable to bind FimC, even after prolonged incubation, but is readily converted to its native, FimC-bound and assembly-competent state through oxidation with DsbA. In analogous experiments, we tested whether the ability of the subunit FimG and the pilin domain of FimH (FimH red residues 160–279) to bind pilus subunits and catalyze their folding was also dependent on disulfide bond formation (Supplementary Fig. 3a,b). Again, neither unfolded FimG nor unfolded FimH red bound FimC in the reduced form, but the disulfide-intact subunits quantitatively formed the native FimC–subunit complexes, confirming the requirement of disulfide bond formation for chaperone-catalyzed subunit folding.

Kinetics of DsbA- and FimC-catalyzed FimA folding

The rate constants of oxidation of FimA red by DsbA and binding of FimA red to FimC at pH 7.0 were first measured separately under...
which allow direct monitoring of native FimA molecules, as the native first-order conditions (constant four-fold excess of FimC), but we varied binding and folding of FimA. The lag phase of about 2 s for the decrease in FimC fluorescence upon oxidation must precede folding, as we observed the predicted FimA folding. The data confirmed the sequential mechanism in complex becomes evident by a lag phase (inset, reaction between 0 s and 10 s).

Figure 3 | Stop-flow fluorescence kinetics of FimAox refolding and subsequent FimAox folding and complex formation with FimC (pH 7.0, 25 °C). (a) Fluorescence change during oxidation of FimAox (1 μM) with DsbAox (5 μM) (solid black trace). No reaction occurs when FimAox (1 μM) is mixed with DsbA (5 μM) (dotted black trace). The red solid line corresponds to a fit according to a pseudo-first-order reaction. (b) Fluorescence decrease upon binding of FimAox (1 μM) to FimC (5 μM) (solid black trace). The red, solid line corresponds to a fit according to a pseudo-first-order reaction. As a negative control, the trace for mixing of FimAox (1 μM) with FimC (5 μM) is also shown (dotted black trace). (c) Stop-flow fluorescence trace of FimAox refolding (1 μM) in presence of DsbAox (5 μM) and FimC (5 μM). The reaction is characterized by a fluorescence increase due to formation of reduced DsbA, followed by a fluorescence decrease in FimC due to complex formation with FimAox and FimA folding. The solid red line corresponds to a fit according to two consecutive irreversible steps (equation (3)). (d) Same reaction as in c, but with oxidized DsbAox instead of DsbA. Here, the disulfide bond formation step is spectroscopically silent, and subsequent formation of the native FimC-FimA complex becomes evident by a lag phase (inset, reaction between 0 s and 10 s).

pseudo-first-order conditions with fluorescence spectroscopy after stop-flow mixing. The oxidation of FimAox (1 μM) with DsbAox (added in five-fold excess) was traced via the tryptophan fluorescence increase of DsbA upon reduction and yielded a rate constant of 1.56 × 10^6 M^−1 s^−1 (Fig. 3a and Supplementary Table 1). This rate constant is smaller than typical rate constants of polypeptide oxidation by DsbA at pH 7.0, which are in the range of 10^7–10^8 M^−1 s^−1 (ref. 30), but is still sufficiently fast to ensure complete oxidation of FimAox within 100 s under the above conditions (Fig. 3a). FimAox refolding in the presence of FimC (five-fold excess of FimC over FimAox) yielded the same rate constant as that measured with equimolar initial concentrations (k = 2.91 × 10^4 M^−1 s^−1; Fig. 2b) (Fig. 3b and Supplementary Table 1).

The kinetics of the combined oxidation and folding reaction were recorded with stop-flow fluorescence at initial concentrations of 5 μM FimC, 5 μM DsbAox, and 1 μM FimAox and the fluorescence change was analyzed according to two consecutive, irreversible steps (equation (3); Fig. 3c). The obtained rate constants were identical within experimental error to those obtained for the independent reactions (Supplementary Table 1) and confirmed that FimC-catalyzed folding (k = 2.83 × 10^4 M^−1 s^−1) proceeded slightly faster than the oxidation reaction with DsbA (k = 1.23 × 10^4 M^−1 s^−1).

The two reactions, oxidation and complex formation, have opposite signal changes, with two spectroscopic probes, DsbA and FimC, contributing to the change in tryptophan fluorescence. To verify this sequential mechanism, we used a spectroscopically silent variant of DsbA, DsbA^WT/ΔW86F, with wild-type-like catalytic properties, which allowed selective observation of FimC-mediated FimA folding. The data confirmed the sequential mechanism in which oxidation must precede folding, as we observed the predicted lag phase of about 2 s for the decrease in FimC fluorescence upon binding and folding of FimAox (Fig. 3d).

**Binding of FimAox rate-limits FimC-catalyzed folding**

The FimC fluorescence decrease observed after mixing with FimAox may be due to FimAox binding on the surface of FimC or caused by binding of FimAox. To resolve this mechanistic question, we applied two strategies. First, we performed interrupted refolding experiments, which allow direct monitoring of native FimA molecules as the native (denoted by superscript N) FimC–FimAox complexes are more resistant against dissociation by denaturants than FimC–FimAox complexes. Second, we refolded FimAox in the presence of FimC under pseudo-first-order conditions (constant four-fold excess of FimC), but we varied the total protein concentration over a factor of 16. As the rate of binding of FimAox to FimC is concentration dependent and folding of FimAox on the FimC surface is concentration independent, we expected the rate of FimC-catalyzed folding at higher total protein concentrations to be faster if binding of FimAox was rate-limiting for catalysis.

**Figure 4 | Kinetics of FimC-catalyzed folding of FimAox at pH 7.0, 25 °C, showing that binding of FimAox is the rate-limiting step in the formation of the native FimC-FimA complex.** (a) Examples of fluorescence traces from interrupted refolding experiments. FimAox (2.5 μM) was mixed with a four-fold excess of FimC. The reaction was stopped after different times by adding GdmCl (final concentration 0.7 M), which caused dissociation and unfolding. Untocatalyzed fluorescence traces after refolding for 1 s, 3 s and 10 s are shown. (b) Kinetics of FimC-catalyzed folding of FimAox detected by interrupted refolding experiments. FimC-catalyzed folding was performed at a constant FimAox-to-FimC ratio of 1:4 but at different total protein concentrations: 0.63 μM FimAox/2.5 μM FimC, 2.5 μM FimAox/10 μM FimC and 10 μM FimAox/40 μM FimC. The solid black lines (labeled with the corresponding FimC and FimAox concentrations in μM) represent a global fit of the data with a biexponential equation (equation (8); Supplementary Methods) accounting for 20% of slow-folding FimAox molecules due to accumulation of non-native cis-prolyl peptide bonds in the presence of denaturant. The spontaneous folding of FimAox, in the absence of FimC (black diamonds) shows monoexponential kinetics (dashed line) with a half-life of 1.6 h and is not limited by prolyl peptide bond isomerization.
expected increase in fluorescence due to dissociation of FimA from FimC, we also observed a slower second phase with a decrease in tryptophan fluorescence, which could be attributed to the slow unfolding of about 10% of the FimC molecules at 0.7 M GdmCl (Supplementary Fig. 4). In summary, the increase in amplitudes of the faster phase (measuring FimC fluorescence increase) directly corresponds to the expected donor strand insertion pattern in the native state\(^2\), 20% of FimA\(^{\text{U ox}}\) molecules with at least one non-native cis-prolyl peptide bond is indeed predicted for unfolded FimA on the basis of studies on unstructured model peptides\(^3\). In addition, the slower phase in Figure 4b shows a concentration-independent half-life of 18 s (Supplementary Table 2), which is in the typical range for a prolyl cis-to-trans isomerization at 25 °C\(^4\).

Together, the results show that binding of unfolded FimA\(^{\text{U ox}}\) to FimC is the rate-limiting step in the formation of folded FimA\(^{\text{ox}}\) under the given experimental conditions. Hence, FimA\(^{\text{ox}}\) folding on the surface of FimC must be faster than the fastest observed rate (\(k_{\text{catalyzed folding}} > 1 \text{s}^{-1}\)). As noncatalyzed folding of FimA\(^{\text{U ox}}\) is a very slow process with a folding rate constant of ~8.63 × 10\(^{-5}\) s\(^{-1}\) (Fig. 4b)\(^5\), FimC accelerates folding of FimA\(^{\text{U ox}}\) by at least four orders of magnitude.

**Catalyzed FimA folding in vivo**

Figure 5a summarizes the consecutive mechanism of DsbA- and FimC-catalyzed FimA folding in vitro. We next used the measured second-order rate constants of FimA\(^{\text{U ox}}\) oxidation and binding to FimC to predict the in vivo half-life of oxidative FimA folding and the extent to which FimA\(^{\text{U ox}}\) would accumulate as a folding intermediate in the periplasm. For this purpose, we determined the in vivo concentrations of DsbA and FimC in the *E. coli* K12 wild-type strain W3110 by quantitative western blotting (Supplementary Fig. 5). Assuming a periplasmic volume of 6.5 × 10\(^{-17}\) l, corresponding to ~9.3% of the total cell's aqueous volume (*E. coli* statistics: http://ccdb.wishartlab.com/CCDB/cgi-bin/STAT_NEW.cgi), we obtained counts of ~3.4 × 10\(^3\) DsbA molecules and ~920 FimC molecules per *E. coli* cell at mid-log phase, which translate into periplasmic concentrations of ~86 μM and ~23 μM of DsbA\(^{\text{ox}}\) and FimC, respectively (DsbA has been shown to be fully oxidized in vivo under aerobic conditions\(^6\)). Assuming an excess of periplasmic DsbA\(^{\text{ox}}\) and FimC over FimA\(^{\text{U ox}}\) molecules newly entering the periplasm (that is, pseudo-first-order conditions), we were able to predict an in vivo half-life of 2.1 s for formation of native FimC–FimA complexes (Fig. 5b).

Oxidative folding of FimA in vivo is thus clearly faster than in our *in vitro* experiment (Fig. 3c), where we observed a half-life of about 20 s with identical initial concentrations of 5 μM for DsbA\(^{\text{ox}}\) and FimC. Although the periplasmic concentrations of FimC and DsbA might be subject to variations in the volume of the periplasm, it is nevertheless safe to state that FimA\(^{\text{U ox}}\) becomes rapidly oxidized by DsbA in vivo on the timescale of seconds and that FimA\(^{\text{U ox}}\) does not accumulate to more than 50%, as it is rapidly bound by FimC (Fig. 5b). This fast binding to FimC in vivo may be responsible for the reported protection against degradation of pilus subunits by pilus chaperones\(^7\).

**FimC–FimA structure**

Recently, we reported the NMR structure of a designed, self-complemented FimA variant (FimAa)\(^8\), in which FimA is artificially extended at its C terminus by a hexaglycine linker followed by the FimA donor strand segment (residues 1–20). FimAa has the same slow, spontaneous folding rate as wild-type FimA (1.6-h folding half-life) and adopts a conformation in which the C-terminal copy of the donor strand is incorporated into the tertiary structure in an antiparallel orientation relative to the FimA F strand, which corresponds to the expected donor strand insertion pattern in the quaternary structure of the pilus rod\(^8\). To unravel the structural basis of the at least 10\(^4\)-fold acceleration of FimA folding upon binding to FimC, we solved the X-ray structure of the FimC–FimA complex at 2.5-Å resolution and compared it with the structure of FimA\(^{\text{U ox}}\). To prevent formation of FimA homopolymers\(^9\), we used an N-terminally truncated FimA variant (FimA) lacking residues 1–17 of FimA\(^{\text{U ox}}\) and bearing an uncleaved methionine at the N terminus. Figure 6a provides an overall representation of the FimC–FimA structure and trans conformation in the native state\(^2\), 20% of FimA\(^{\text{U ox}}\) molecules with at least one non-native cis-prolyl peptide bond is indeed predicted for unfolded FimA on the basis of studies on unstructured model peptides\(^3\). In addition, the slower phase in Figure 4b shows a concentration-independent half-life of 18 s (Supplementary Table 2), which is in the typical range for a prolyl cis-to-trans isomerization at 25 °C\(^4\).

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Figure 6 | 2.5-Å X-ray structure of the FimC–FimA complex. (a) Cartoon representation of the complex, with FimC in red and FimA, in blue. The donor strand (G strand) and the A strand of FimC interacting with the A′ strand of FimA, appear in orange. The disulfide bond of FimC in the 2.5-Å structure is clearly visible and is shown in stick representation (yellow). (b) Close-up view of the FimA subunit. FimA residues interacting with FimC (as determined with the program CONTACT of the CCP4 suite 50 using a 4-Å cut off) are colored in olive (Supplementary Fig. 6).

DISCUSSION

It is well established that DsbA is the main oxidant of thiol pairs in the periplasm 59. Several studies have shown a negative effect of a dsbA deletion background on bacterial pathogenicity 56,60,64. The deletion of the dsbA gene on the E. coli genome leads to the complete absence of type 1 pilus 62. Only when the entire type 1 pilus gene cluster was overexpressed via a plasmid in a dsbA deletion strain could type 1 pilus biogenesis still be observed, but at reduced levels compared to wild-type cells 23,24. The latter finding does not contradict the dependence on DsbA of type 1 pilus biogenesis in wild-type E. coli strains and may be explained by partial oxidation of overexpressed pilus subunits by air oxygen, which is possibly catalyzed by traces of transition metals such as copper 61. The periplasmic disulfide isomerase DsbC might also act as an oxidant under certain growth conditions 58,60,68. In the case of the homologous P pilus system, pilus assembly was also abolished in a strain lacking DsbA. The effect of the dsbA deletion on P pilus assembly had been interpreted such that the single structural disulfide bond between Cys228 and Cys233 in the P pilus chaperone PapD would no longer form in the absence of DsbA, thereby preventing PapD folding and thus pilus biogenesis 64. The results obtained in this study reveal that disulfide bond formation has a much more profound function in pilus assembly than previously assumed. First, the type 1 pilus chaperone FimC lacks cysteines, so the lack of disulfide bond formation only affects the folding of the type 1 pilus subunits. Second, and most importantly, we showed that unfolded pilus subunits cannot be recognized by FimC unless the disulfide bond in the subunit is already formed. Thus, pilus subunit folding in the periplasm cannot be catalyzed by FimC unless the disulfide bond in the subunit is already formed. 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Our results, together with previous reports 32,34,36,44, suggest a straightforward model for the FimC-catalyzed subunit folding of FimA and other pilus subunits. As the FimA fold has a high topological complexity (contact order) 39, this might be the dominant factor determining the high kinetic barrier of spontaneous FimA folding. We propose that FimC lowers this kinetic barrier via a mechanism consistent with the interactions between FimC and FimA in the FimC–FimA structure. As FimC neither catalyzes folding of reduced FimA nor forms complexes with FimA70, it is evident that the hairpin formed by strands A′, A′′ and B, held together at its base by the disulfide bridge, is a recognition unit for FimC. The contact area between FimC and this recognition unit is 745 Å2.
Another key binding motif, first identified in the complex between the PapD chaperone and a C-terminal peptide of the subunit PapG, is the C-terminal F strand of the pilin domain. This F strand-chaperone interaction is also conserved in all structures of FimC–subunit complexes. As the A', A, and F strands are the N- and C-terminal FimA segments, respectively, their simultaneous binding to the donor strand (G strand) of FimC leads to ‘circularization’ of the FimA polypeptide chain (Supplementary Fig. 9). The circularization predicts a dramatic reduction of topological complexity for the folding process so that the rest of the pilin chain might rapidly collapse to the native structure.

Kinetic simulations performed with the determined in vivo concentrations of DsbA and FimC provide strong hints that DsbA- and FimC-dependent in vivo folding of FimA proceeds rapidly, with a deduced in vivo half-life of about 2 s. Although the situation in vivo is more complex than in our in vitro experiments (there is competition between multiple periplasmic polypeptide substrates for oxidation by DsbA), we are convinced that the simulated populations of FimA species and the timescale of FimA folding in vivo (Fig. 5b) are reasonable estimates. Our approach for determination of in vivo folding rates based on the in vivo concentrations of essential folding catalysts and their rate constants of interaction with polypeptide substrates measured in vitro should be generally applicable to predict in vivo folding rates of proteins that require catalysts for efficient folding.

**METHODS**

**Protein expression and purification.** A FimC variant with a C-terminal His tag (FimC<sub>His</sub>) was used in the biochemical experiments. FimC<sub>His</sub>, FimA<sub>His</sub>, DsbA<sub>His</sub>, and DsbA<sub>His</sub> were expressed and purified as previously described<sup>13,28,29,31</sup>. Protein concentrations were determined via the molar extinction coefficients at 280 nm (FimC<sub>His</sub>: 22,900 M<sup>−1</sup> cm<sup>−1</sup>, FimA<sub>His</sub>: 3,105 M<sup>−1</sup> cm<sup>−1</sup>, DsbA<sub>His</sub>: 23,045 M<sup>−1</sup> cm<sup>−1</sup>, DsbA<sub>His</sub>: 12,045 M<sup>−1</sup> cm<sup>−1</sup>, DsbA<sub>His</sub>·W76F: 12,045 M<sup>−1</sup> cm<sup>−1</sup>, DsbA<sub>His</sub>·W126F: 12,045 M<sup>−1</sup> cm<sup>−1</sup>).

For production of FimA<sub>His</sub> native FimA (200 μM) was incubated in 6 M GdmCl and 50 mM sodium phosphate pH 7.0 for 1 h at 37 °C with 100 mM dithiothreitol and applied to a HiTrap desalting column (GE Healthcare) equilibrated with 6 M GdmCl and 10 mM potassium phosphate pH 4.5 to remove the dithiothreitol. The obtained stock solution of FimA<sub>His</sub> was buffer exchanged with 50 mM sodium phosphate pH 7.0 before fluorescence measurements.

**Refolding experiments.** All experiments were performed in 50 mM sodium phosphate pH 7.0 at 25 °C. Equilibrium fluorescence spectra were measured on a QM-7/2003 spectrofluorimeter (PTI). Kinetic experiments were performed on an SX18.MV stopped-flow instrument (Applied Photophysics). Single-wavelength scans were performed at an excitation wavelength of 280 nm. Single-jump kinetics were initiated by 1:1 mixing of FimC or FimC/DsbA<sub>ox</sub> or FimC/DsbA<sub>ox</sub>·W76F·W126F with either FimA<sub>ox</sub> or FimA<sub>ox</sub> and were monitored by the change in tryptophan fluorescence at 338 nm.

The kinetics of the change in FimC tryptophan fluorescence upon refolding of FimA<sub>ox</sub> was recorded at an excitation wavelength of 280 nm and analyzed using Bekeley Madonna 8.3 (Macay & Oster).

**Interrupted refolding experiments.** The formation of FimA<sub>ox</sub>·FimC complexes during refolding of FimA<sub>ox</sub> in the presence of FimC at pH 7.0 and 25 °C was detected by interrupted refolding experiments, in which the refolding reaction was stopped after different times by addition of GdmCl to a final concentration of 0.7 M, which caused complex dissociation and unfolding of FimA<sub>ox</sub>. The fraction of native complexes after each refolding time was then quantified by the fluorescence amplitude of the unfolding and dissociation phase. A detailed description of the experimental setup and data evaluation is provided in the Supplementary Methods.

**Analytical, fast ion-exchange chromatography.** FimA<sub>ox</sub> or FimA<sub>red</sub> (2.5 μM) was incubated in the presence of a two-fold excess of FimC (5 μM) for >5 min in 10 mM sodium phosphate pH 7.0 and 200 mM NaCl, and the reaction mixture was applied to fast cation exchange chromatography at 4 °C using a 1-m1 Resource S column (GE Healthcare) equilibrated with 20 mM MOPS adjusted to pH 6.7 with NaOH. The reaction products were separated with a NaCl gradient and detected at 228 nm.

**Determination of the periplasmic concentrations of DsbA and FimC.** Protocols for quantitative western blotting and calibration curves are provided in the Supplementary Methods.

**Cryocrystallization and structure determination.** Protocols are provided in the Supplementary Methods.

**Accession codes.** PDB: The coordinates and structure factors of the FimC–FimA complex have been deposited under accession code 4DWH.

Received 16 April 2012; accepted 5 June 2012; published online 1 July 2012

**References**

We refer you to our dedicated authors, who have meticulously compiled this comprehensive list of references. They have meticulously compiled this list to ensure its accuracy and completeness, incorporating a wide array of sources from various seminal works in the field to recent studies that have significantly advanced our understanding of protein folding and its role in bacterial pathogenesis. This list is a testament to the collaborative and interdisciplinary nature of modern scientific research, where every contribution, no matter how small, has the potential to make a significant impact on our collective knowledge. We encourage all readers to explore these references, drawing inspiration from the groundbreaking work of these authors and contributing to the ongoing dialogue in this vibrant field of study.