Adhesion mechanisms of curli subunit CsgA to abiotic surfaces

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Curli fibers are functional amyloids that play a key role in biofilm structure and adhesion to various surfaces. Strong bioinspired adhesives comprising curli fibers have recently been created; however, the mechanisms curli uses to attach onto abiotic surfaces are still uncharacterized. Toward a materials-by-design approach for curli-based adhesives and multifunctional materials, we examine curli subunit adsorption onto graphene and silica surfaces through atomistic simulation. We find that both structural features and sequence influence adhesive strength, enabling the CsgA subunit to adhere strongly to both polar and nonpolar surfaces. Specifically, flexible regions facilitate adhesion to both surfaces, charged and polar residues (Arg, Lys, and Gln) enable strong interactions with silica, and six-carbon aromatic rings (Tyr and Phe) adsorb strongly to graphene. We find that adsorption not only lowers molecular mobility but also leads to loss of secondary structure, factors that must be well balanced for effective surface attachment. Both events appear to propagate through the CsgA structure as correlated motion between clusters of residues, often H-bonded between rows on adjacent β strands. To quantify this, we present a correlation analysis approach to detecting collective motion between residue groups. We find that certain clusters of residues have a higher impact on the stability of the rest of the protein structure, often polar and bulky groups within the helix core. These findings lend insight into bacterial adhesion mechanisms and reveal strategies for theory-driven design of engineered curli fibers that harness point mutations and conjugates for stronger adhesion.

INTRODUCTION

Adhesive, persistent, and metabolically efficient, bacterial biofilms often present significant challenges in healthcare, naval, and industrial settings because of their ease of formation and difficulty in removal (1–5). Yet, biofilms have also been harnessed for applications, such as water filtration and bioreactors (6, 7) and biological fuel cells (8). The biofilm extracellular matrix (ECM) protects the bacteria, largely dictates overall mechanical properties, and can vary in composition and structure across different species and strains (9, 10). So far, continuum-scale properties of biofilm adhesion have been investigated (11, 12), but the molecular-scale mechanisms are yet to be determined and are necessary to accurately represent these properties. Within Escherichia coli biofilms—which are particularly well studied—curli fibers are one of the major peptide-based components that make up the ECM (13).

Curli grow into fibers on the surface of E. coli bacterial cells, and seven “curli-specific genes” (csg) are involved in their expression (14, 15). Curli are mostly made up of CsgA, a β helix of five repeat units. CsgA self-assembles on the cell surface (16) and inherently fibrillates (17), and curli assembly can be modulated by chemical inhibitors, in addition to protein chaperones (18). Although curli nano-fibers are amyloids, they are functional and serve distinct biological purposes, contrasting from well-known dysfunctional amyloids that aggregate into plaques in diseases, such as Alzheimer’s (19). For example, β-amyloid has been widely studied computationally (20, 21) and experimentally (22–24) in regard to amyloid adsorption and fibrillation. Similarly, other amyloids, such as lysozyme and β-lactoglobulin, have been well described at interfaces (25–27) and used in composites to create hybrid materials with remarkable properties (28–31), while characterization of curli is still emerging. Still, curli have the characteristic cross-β hydrogen–bonded structure of amyloids commonly associated with proteinaceous fibers having outstanding stiffness and strength. Experiments mapping biofilm mechanics have shown that curli provide stiffness and mechanical homogeneity within their biofilms (32) and serve to reinforce the biofilm structure (33–35), making up most of the insoluble biofilm matrix (36, 37). Curli also play a key role in invading host domains by activating contact proteins and binding with fibronectin for the internalization of bacteria, which is enhanced by increased curli production and further heightened for pathogenic strains (38–42).

Additionally, evidence from genomic and mechanical characterizations of biofilms suggests that curli are critical in enabling bacterial adhesion to many abiotic surfaces through formation of adhesion fimbriae (43, 44). Studies with varying curli production indicate that the fibers are critical for strong adhesion (34, 45), and increased curli expression has been shown to result in pellicles with increased surface elasticity and strength (43). Curli overproduction has been shown to substantially increase adhesion, and attachment strength has been noted to vary by surface type: the strongest interactions were seen for hydrophobic plastics, relative to hydrophilic metals or glass (45, 46). Still, the fundamental mechanisms that allow curliated biofilms to achieve this outstanding adhesion are yet unknown.

Whereas the structures of many curli-specific genes are becoming realized via computation (47–49), the main curli component, CsgA, contains a repetitive structure that creates a challenge in structural characterization. Experimental techniques, such as solid-state nuclear magnetic resonance (ssNMR) spectroscopy and computational models based on multiple sequence alignment, have begun to capture features of the CsgA structure (47, 50, 51). The structural features—such as repetitive β strands—found in these studies corroborate each other, as well as structural predictions for the Salmonella analog AgfA (47, 52).

In addition, curli fibers have already been successfully combined with various conjugations to produce materials with enhanced properties.

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Adhesive strength was enhanced through conjugation with the mussel foot protein, and curli fibers were also interfaced with inorganic nanoparticles and quantum dots (53–55). Despite recent advances in developing novel materials with curli, the cause behind their adhesive strength remains a mystery. Here, we aim to first uncover the molecular mechanisms behind adhesion of the CsgA subunit. So far, qualities that contribute to overall adhesion, and whether these contributions are dictated by residue chemistry, shape, size, or other features, are yet to be clarified. The adsorption of small proteins to abiotic surfaces using all-atomistic and coarse-grain models in explicit solvent or other media has been computationally quantified through Steered Molecular Dynamics (SMD) and energetic analysis, including the “two-box” and “four-box” methods, as well as by visualization and conformational analysis (20, 21, 56–61). Here, we use an explicit, all-atom approach in assessing interaction energy per unit area between the CsgA and each surface and the role of residue features and motion during the adsorption process. Our approach focuses primarily on enthalpic interactions governing adsorption, which call for an atomistic methodology that can ascertain residue-specific features that promote adhesion and should be conserved while scaling up the larger model of a curli fiber.

To address this, we present fully atomistic molecular dynamics simulations that examine CsgA surface interactions with model hydrophobic and polar surfaces. We first investigate the role of residue-specific features in adhesion, such as the impact of side-group chemistry and geometry on adsorption onto different surfaces. As the CsgA subunit undergoes structural changes during the adsorption process, we also study structural events that impact stability. These are analyzed in regard to how they propagate throughout the structure in correlated groups of residues. Residue clusters are then assessed in terms of relative impact on the structural dynamics and stability. We conclude by summarizing our findings on the impact of the primary sequence of CsgA on its structural stability and adhesive properties.

RESULTS
CsgA adhesion to abiotic substrates
CsgA subunit adhesion is first studied to determine (i) surface adhesion energy, (ii) features that facilitate adhesion, and (iii) differences in adhesion mechanisms between polar and nonpolar surface types. Surface adhesion energy is obtained by calculating the total interaction energy between the protein and the surface and normalizing this value by the surface contact area between them. Simulation setup is shown in Fig. 1, and adhesion energies obtained in this fashion are reported in Table 1. CsgA adhesion energy is found to range between −206 and −237 mJ/m² on graphene and between −159 and −196 mJ/m² on silica, underlining that CsgA can bind strongly to both polar and nonpolar surfaces. The adhesion mechanisms are different and depend on substrate chemistry. The SDs in Table 1 quantify how the energy varies in the bound state for each simulation, from which we see that energy variations in the adhered configuration are higher for silica compared to graphene. This is because residues attaching to graphene do so through van der Waals interactions, allowing the molecule to adhere with smaller energy variations. Alternatively, the energy landscape is rougher for silica due to electrostatic peptide-surface interactions. In this case, initial contact anchors the molecule onto the surface; this leads to energy fluctuations as the molecule pivots to maximize its favorable polar surface interactions through sampling local configurations. Simulations reveal that CsgA residues (sequence

**Table 1. Adhesion energy.** Adhesion energy between the protein subunit and both surfaces, organized by initial orientation of the protein on the surface. Adhesion energy is calculated as the interaction energy with the surface as a function of surface contact area (described in Materials and Methods).

| Orientation | Adhesion energy (mJ/m²) |
|-------------|------------------------|
|             | Graphene               | Silica                  |
| 0°          | −236.97 ± 16.69        | −159.01 ± 27.79         |
| 90°         | −230.06 ± 12.51        | −180.19 ± 30.39         |
| 180°        | −205.82 ± 19.53        | −195.52 ± 21.85         |
| 270°        | −215.66 ± 11.64        | −190.68 ± 42.58         |

**Fig. 1. Schematic of system setups.** The CsgA protein subunit (A) is shown with both termini labeled above each substrate (B) and in four different starting configurations (C). Idealized graphitic and silica surfaces were used, resulting in a total of eight systems that we characterized to obtain interfacial properties. Setups with various orientations were produced by rotating the subunit about the last β strand near the C terminus by 0°, 90°, 180°, and 270°, so that either a β sheet face or a terminus is exposed to the surface. The subunit was placed so that the bottommost atom was 7 Å above the surface.
The data presented in Fig. 2 reveal that the strongest interactions on graphene are distinguished by residues with prominent aromatic side groups. The difference in surface interaction energy between the two surfaces is most heightened for six-carbon rings in Tyr and Phe, which share a common hexagonal motif with graphene (Fig. 3). These aromatic residues promote hydrophobic interactions that are also presumably significant in the formation of amyloid fibers (62). Local protein flexibility—often driven by the loss of secondary structure—facilitates side-chain reorientation to achieve minimum energy configurations on the surface. Reorientation for optimal alignment can be seen in Fig. 3A for the case of tyrosine on a graphitic surface. While flush against the graphene surface, moving from an AA to AB alignment lowers the instantaneous interaction energy of Tyr by 3.72 kcal/mol, agreeing with earlier studies on optimal stacking of aromatic rings (63). Other studies examining binding energies of amino acids on graphene sheets identify Tyr as among the strongest, as well as Trp (57–59). Tyr is unable to achieve this flush configuration with silica, as the distal hydroxyl group can even incur unfavorable repulsive electronegative forces with polar groups on the silica surface (fig. S2). These findings highlight the role of specific residues in achieving strong adhesion to a substrate. We note that, in the case of multifaceted large molecules, such as CsgA, calculating average interaction energy for each residue is sensitive to the position of the residue in the protein structure and the adsorption configuration. Specifically, having larger numbers of residues situated far from the substrate will reduce the reported interaction energy when the data are averaged over all residues. Contributions within CsgA are influenced by access to the surface, which can vary on the basis of neighboring amino acids, flexibility, and overall protein orientation.

Whereas interactions between CsgA and graphene come solely from van der Waals interactions, the exposed charges on silica contribute to both repulsive and attractive interactions with the protein. These interactions can enhance attractive strength for compatible residues, namely, positively charged side chains in Arg and Lys and polar side chains in His, Tyr, Thr, and Gin, which have relatively high proportions of attractive energy from electrostatics (fig. S3A). The residues Arg and Lys have also been shown to anchor on silica nanoparticle surfaces through solution NMR data of smaller peptides (64). Whereas electrostatic contributions make up, on average, 81% of the total interaction energy between Lys and silica, interactions with hydrophobic residues come mostly from van der Waals contributions. In the case of Leu, 97.7% of the total interaction energy with silica comes from van der Waals contributions (fig. S3A). Polar aromatic residues, such as His and Tyr, show potential in using both chemistry and geometry to adhere strongly to both surface types and are the only residues with mean interaction energy measured below ~4 kcal/mol for both surfaces. Although polar and positively charged CsgA residues may have stronger electrostatic interactions with silica, exposed partial charges on the substrate surface also induce unfavorable repulsive interactions with the negatively charged residues and polar side chains situated poorly on the surface. Maximum repulsive energies between the CsgA residues and silica can be found in fig. S3B and show that polar and negatively charged residues encounter the strongest repulsion with the polar surface, followed by certain hydrophobic residues. Reorientation to optimize surface interactions yields even larger energetic changes than geometrically aligning similar side chains on the graphene surface.

We see that simply rotating an Asn side chain decreases the instantaneous interaction energy between that residue and the surface by 14.68 kcal/mol

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**Fig. 2. Surface interaction energy grouped by residue moiety.** Energies comprise electrostatic and van der Waals interactions and are taken from the last 20 ns of each trajectory. Energetic values are only included when a given residue has at least one atom within a 5.5 Å cutoff distance of the surface. These values are also averaged across orientations and compared between surface types. Energies are normalized by the number of each residue in the CsgA structure and are grouped by feature. Interaction energies with graphene are shown in solid bars, and silica interaction energies are represented with diagonal stripes. Residues Ile and Met are excluded from this plot, because they are situated within the helix core and do not contact the surface. Polar residues and positively charged residues show the strongest preferences for silica relative to graphene. Negatively charged residues incur repulsive energies with silica, and charged residues, in general, produce weaker interaction energies with graphene. Aromatic six-carbon residues, such as tyrosine (Tyr) and phenylalanine (Phe) exhibit strong preferences to graphene relative to silica because of compatible geometry. Polar aromatic residues, specifically His and Tyr, significantly contribute to adhesion energy for both types of surfaces and have the largest overall surface interaction energies for both cases.
Next, we analyze how the local motion of side chains promotes adhesion by facilitating transitions to minimum energy configurations on the surface. CsgA preferentially interfaces flexible turn and termini regions with the surface, rather than the relatively rigid β sheet faces. While approaching the surface, the subunit reorients so that more flexible regions interact with the surface for all initial configurations studied. Snapshots of initial and final conformations of the CsgA can be found in fig. S5. In each trajectory, the adsorbed peptide contained less β sheet structure than its original structure, and this loss of secondary structure was often a result of surface contact. During adsorption, residues near the flexible turns of the helix interacting with the surface tend to lose their secondary structure first, leading to shortened β strands. The β sheet structure was lost to similar degrees for adsorption onto graphene and silica (−13.7 and −11.7% change, respectively). We note that secondary structure loss does not necessarily indicate destabilization. Loss of secondary structure generally induces an increase in B-factor, and therefore flexibility, which allows reorientation of side chains to reach a minimum energy configuration on the surface. B-factor then decreases in response to increases in residue-surface contacts, as the residue motion is damped through “caging” on the surface. Surface caging effects are defined here by a decrease in B-factor in response to surface contact and are present across the entire CsgA structure. Decrease in B-factor per residue in response to surface contact is shown in fig. S6, and the residues within the flexible N terminus can be seen experiencing stronger caging effects than the residues within β sheet faces. Ultimately, the resulting adsorbed configuration balances the enthalpy of surface interactions with the energetic penalty of unfolding. Although unfolding commonly leads to greater residue mobility, we find that destabilization induced by surface contact is compensated by surface caging effects as residue motion is damped by proximity to the surface. These results indicate that the design of surface peptide interactions should achieve a fine balance between flexibility and stability to maximize adhesive capabilities to a surface. If adhesion of self-assembled curli fibrils is considered, preserving the overall β sheet structure upon adhesion is likely necessary to achieve robust adhesive performance.

Correlated motion within CsgA

B-factor is also observed to decrease during CsgA adsorption in residues that do not contact the surface. Therefore, we hypothesize that drops in B-factor are limited not only to the caging of a particular residue but also propagate across the structure through correlated groups of residues. To investigate the propagation of motion throughout the protein, we conduct a correlation analysis (described in Materials and Methods) examining the relationship between the B-factors of pairs of residues. We use this method to observe relationships between the B-factors of intrasheet contacts (peptide bond in the same β strand), interstrand contacts (adjacent β strands), or through-helix contacts (opposite β strands) in the CsgA structure. A description of these B-factor correlation categories and their relative contributions can be found in Fig. 4. We find that changes in B-factor during adhesion events are not only just confined to the changes in secondary structure or surface contacts in a particular residue but are also correlated throughout the protein. The ranking of these relative contributions is as follows: peptide bond > adjacent β strand > opposite β strand > turn. From this analysis, it is clear that more than 80% of the correlated motions occur through peptide bonds or hydrogen bonds between the strands in the β sheets, which constitute a significant portion of the protein structure (Fig. 4C). However, because protein adsorption...
frequently involved the turns of the CsgA structure, nearby covalently bonded residues are perturbed and lose secondary structure first, inducing a change in B-factor. Outside turn regions, interactions with the surface perturb residues across the β strands. This destabilizes the β structure among neighboring β strands. A notable finding is that strong pair correlations are found in the unstructured N-terminal region, apparently because of high mobility. This region also experiences the largest decrease in B-factor during adsorption. Strong B-factor correlations seemingly occur in high-flexibility regions, where motion is more easily transferred across peptide bonds.

In addition to assessing B-factor correlation among pairs of residues, we further investigate motion propagation through groups of residues. Specifically, we examine the loss of secondary structure in response to surface contact, because surface proximity seemingly influences flexibility, and these changes in B-factor are shown to be correlated across pairs of residues. To do this, we correlated time series data of surface interactions (defined by interaction energy) and β sheet membership for each residue. A detailed description can be found in Materials and Methods. This analysis aimed to reveal which amino acids are most strongly perturbed by surface contact and which residues are highly responsive to the motion of other residues. Through these comparisons, similar clusters of residues were identified that showed changes both in B-factor and in β sheet membership for each trial. Groups of residues that responded similarly were located along rows connected by interstrand relationships across neighboring β strands. Groups that showed correlated behavior did so on both surface types. These “clusters” are defined in fig. S7. To simplify data presentation, these correlations were summed by residue group and can be found in Fig. 5.

From the correlation analysis, it is clear that motion transmission throughout the peptide is nonuniform and depends on factors, such as amino acid moiety, size, and location within the structure. Residue groups of interest can be seen on the CsgA structure in Fig. 6. The role of residue location is apparent, because clusters 2 to 5 are located in the center of the rigid β sheet face and show low response to surface contacts in general. Clusters near the edge of the β sheet show increased responsiveness, particularly to the more mobile terminal regions. Strong terminal responsiveness is apparent in clusters 13, 14, and 16 to 18. These groups are located either on the less rigid face of the CsgA structure that readily loses secondary structure (13 and 14) or within the already flexible turn region (16 to 18). It is unknown why cluster 15 shows lower responsiveness than neighboring groups. Clusters 7 to 12, excluding 8, show a heightened sensitivity to perturbations, most strongly in clusters 7 and 12. These residue groups are either near a turn region (7, 9, and 10) or on the same, less rigid face as terminal-responsive groups 14 and 15 (clusters 10 to 12). Particularly susceptible to perturbations, clusters 7 and 9 were shown to lose β sheet structure in all instances of adsorption, involving both surfaces. Aligned within the helix core on opposite β sheet faces, clusters 7 and 9 transfer motion through the Gln residues of cluster 7. Both clusters were also destabilized even when only residues on the opposite side of the protein face contact the surface. Additionally, cluster 1 is shown to respond to residues 149 to 151 in the C terminus. We note that cluster 1, a row of polar Ser residues, sits opposite the bulky row of polar Gln residues in cluster 14. A perturbation in the more flexible terminal end results in the adjustment of opposite or adjacent residues in both clusters to favorably reorient partial charges. These rotations then cascade throughout the protein, because each subsequent residue must reorient after its neighbor.

This correlation analysis serves to examine the CsgA structure and the correlations in motion between pairs and groups of residues. Our
findings show that motion is readily transmitted through peptide bonds, as well as (but to a lesser degree) to neighboring β strands or across the helix core. Additionally, changes in secondary structure within the protein are transmitted across residue groups through hydrogen bond contacts across the structure in response to surface contact. The level of responsiveness of these groups seemingly depends primarily on amino acid location, as well as moiety and size. In this protein, bulky, polar amino acids located near turn regions within the helix core seem to most strongly transmit motion. These results provide important insights into how structural changes—such as B-factor and loss of secondary structure—propagate throughout the CsgA. Knowledge of location and moiety of residue groups exhibiting highly correlated motion could then be used to manipulate CsgA stability through mutations of certain position or type. This outlines a map for potential uses involving deliberate stabilization or destabilization of the structure.

**DISCUSSION**

Although it is believed that curli fibers have exceptional mechanical and adhesive characteristics, the molecular mechanisms enabling these properties remain unexplained. Our investigation regarding the adhesion of the curli subunit CsgA onto ideal hydrophobic and charged surfaces provides new insights into the adhesion mechanisms of curli nanofibers.

We have calculated the curli subunit adhesive strength to be in the range of $-159$ to $-237$ mJ/m$^2$ over all trials conducted (Table 1). These values are an order of magnitude higher than the measured adhesion energy for curliated biofilms [shown to be near 12.5 to 15 mJ/m$^2$ for purified CsgA systems (53)]. This is expected because our system only looks at single subunit interactions rather than a full fiber or fiber network, which should have lower effective surface contact per unit surface area due to the gaps between fibers.

Comparing substrate types, the mechanisms that the CsgA subunit uses to adhere are divergent and specific to the surface they encounter. Through the study of these adhesion mechanisms, we have revealed the importance of both location-based and residue-specific features to achieve high surface energies. Generally, the CsgA subunit prefers to expose flexible regions that are able to reorient and achieve favorable configurations on a surface and do so through partial unfolding and rearrangement, corroborating similar studies (60, 61, 66–68). Additionally, side-chain chemistry and geometry dictate interactions preferentially by surface type (Fig. 2). The polar silica surface interacts...
strongly with compatibly charged positive residues, whereas six-carbon aromatic residues flatten favorably onto the graphene surface (Fig. 3). Polar aromatic residues appear promising in ensuring strong adhesion to both surface types. This analysis identifies key sequence features that facilitate adhesion specific to surfaces, which can guide choices in developing surface-specific adhesives by introducing point mutations in a theory-informed fashion. These results also underline that the versatility of curli fibers enables indiscriminate adhesion to surfaces, which we attribute here to the chemical diversity in the peptide sequence of CsgA subunits.

Our analyses on the structural dynamics during adsorption reveal that surface interactions cause secondary structure loss and lead to caging effects (fig. S6) that must be balanced to maintain stability. Collective motion through structurally related groups in the CsgA leads to propagation of these effects to residues that are not in contact with the surface. Using time series correlation analysis, we determine groups of interrelated residues and identify particularly impactful or susceptible groups (Figs. 5 and 6). These findings signify the presence of potentially high-impact residues that may need to be preserved to create stable mutated CsgA variants through genetic engineering.

We note that the chemical diversity of residue side chains exposed on each face of the CsgA introduces energetic variations between trials. Additionally, specific interactions and propagation of motion near the termini of the isolated monomer are expected to differ from this region in monomers stacked in a fiber. Our future studies will build upon this work to investigate fiber dynamics and adhesive behavior. Although this study focuses on a particular CsgA model, methods to discern adhesion mechanisms and correlated motion can be applied to other amyloid structures to design novel biotic-abiotic interfaces.

The results presented provide key insights on the role of protein features in adhesion. These findings can be applied through strategic manipulation of protein behavior using the features outlined. Point mutations of particular residues may be incorporated to enhance or reduce nonspecific or substrate-specific adhesion. CsgA modifications may be judiciously used at particular locations to tune flexibility (moderate- to high-impact regions) or minimize motion propagation (low-impact regions). Overall, our findings are important for discovering bioinspired adhesives that harness the ability of curli fibers to attach to surfaces, designing surfaces that minimize fouling, and creating abiotic interfaces that couple strongly with structural proteins commonly produced by bacteria.

**MATERIALS AND METHODS**

**Materials**

CsgA subunit models computed using multiple sequence alignment contacts as structural restraints were obtained for use in this study (47). These models comprise a β helix of five repeat units and corroborate structures measured using ssNMR, electron microscopy, x-ray diffraction data, and other computational studies (50, 52, 69). Fixed graphene and (001) α quartz silicon dioxide surfaces were used to model hydrophobic and polar surfaces and were created using the VMD (Visual Molecular Dynamics) Inorganic Builder (70). Although the degree of surface ionization on silica can influence surface properties and adsorption mechanisms (71–74), we selected a single silica model to represent a charged surface. The silica surface has exposed oxygen atoms (negative charge) and silicon atoms (positive charge), but no added hydroxyl groups, representing an idealized surface.
All simulations were all-atomistic and conducted using the NAMD (Nanoscale Molecular Dynamics) simulation package (75). All systems were solvated in explicit water solvent using the CHARMM version of the TIP3P model (76). The surface and the protein were both modeled using established parameterizations based on the CHARMM force field, and graphene was modeled as sp² carbons (77, 78). Long-range electrostatics were calculated using the particle mesh Ewald technique (77). Simulations were conducted at 300 K, using a 1-fs time step at a constant pressure of 1 atm in the isothermal–isobaric (NPT) ensemble. Periodic boundary conditions were used in all directions in each simulation, with a minimum of 15 Å padding on each side to prevent the protein from interacting with its image. The total box height is 70 Å, which is large enough to prevent the CsgA from interacting with the surface’s image.

Simulation setup of CsgA on a surface
Simulations were conducted with the left-handed CsgA subunit beginning in four different orientations above each surface to give access to all sides of the protein. Schematics illustrating the system setup can be found in Fig. 1. The subunit was placed uniformly in each trial so that the bottommost atom was 7 Å above the surface. This distance was chosen to provide a reasonable time scale for adsorption without hindering the rotation of the protein, which allows surface interactions with different sides of the protein. These systems underwent energy minimization for 50,000 steps and were allowed to interact with the surface over the course of a 60-ns trajectory. Here, protein behavior was investigated in regard to the role of structural and residue-specific features in adhesion, as well as correlated motion throughout the CsgA. In total, over 480 ns of simulations have been run to model this system.

Energetic analysis of CsgA on a surface
Energies between the CsgA subunit and the surface were computed using the NAMDEnergy tool. These comprise nonbonded interactions, including electrostatic and van der Waals contributions. Contact energy was calculated by the total protein-surface interaction energy in regard to the contact area between them. Contact area was computed by calculating the total projected area of atoms within a 5.5 Å cutoff distance of the surface based on the size of their van der Waals radius. Surface adhesion energy was calculated throughout the course of the adsorption, and the converged average value over 20 ns is used. For energetic analysis by amino acid, energies are averaged across all trial orientations per surface and normalized by occurrences within the protein, representing the average contribution of each residue.

Time series analysis
Time series analysis was conducted for B-factor, surface interactions, and secondary structure measurements across all trajectories. B-factor was calculated to examine the loss of B-factor during adsorption, as well as correlations in B-factor between groups of residues. This was conducted using the b-factor TCL script developed by A. Oakley. For B-factor loss upon adsorption, B-factor was calculated for each atom, and for each residue, the average B-factor of all atoms within the residue was calculated. This is done so that the change in B-factor during adsorption represents each atom interacting with the surface. To study the correlations in the changes in B-factor between residues, the B-factor was calculated for the β carbon of each residue and the hydrogen atoms in glycine. β carbons are chosen here as they are more representative of the flexibility of the side chain, whereas side group atoms may be highly influenced by adsorption, and α carbon B-factor is more indicative of backbone movement, which can be captured by secondary structure.

The time series data for secondary structure were constructed by calculating the percent time a residue was within a β strand for 250-ps windows across the entire trajectory. The time series data for secondary structure and B-factor for each residue were assessed in combination with the time series data for surface interactions. Here, β sheet structure was calculated using the STRIDE (structural identification) algorithm (79). The number of surface interactions was calculated as the number of interactions between each protein atom and each surface atom within 5.5 Å, and the time series data were constructed as the total number of interactions within 250-ps windows over the entire trajectory.

B-factor correlation analysis
Correlation analysis was conducted by comparing the B-factor of each residue to all others in all possible pair combinations through linear regression of B-factors in subsequent time windows. Feasible interaction pairs were determined using a protein contact map to define pairs by a peptide bond, by a hydrogen bond between neighboring β sheets, or by residues opposite each other across the β helix. For each category of feasible interaction pairs, the correlation coefficients of B-factor were summed.

Collective motion analysis
The time series data for β sheet membership, surface contacts, and B-factor were then used to further study collective motion among residues. Residues were grouped across clusters on the basis of hydrogen bonds across the β sheet faces based on the ssNMR structure (50) to identify groups of residues that are more susceptible to losing the β sheet structure. Residues within the clusters exclude the N-terminal region that is outside the helix. The correlation between the surface-contact and β sheet time series data is summed within each cluster, and the sums are normalized by the number of residues in the stack. For each residue, the surface interaction time series data were cross-correlated with the corresponding B-factor time series data. These data are represented in Fig. 5, where the value of each pixel is the sum of the correlations between a particular residue contacting the surface and the loss in β sheet structure in all the residues making up that cluster, normalized by maximum correlation.

SUPPLEMENTARY MATERIALS
Supplementary material for this article is available at http://advances.sciencemag.org/cgi/content/full/2/11/e1600998/DCl

Sequence diagram and correlated motion groups
Interactions between tyrosine and silica
Energetic contributions summed by type on silica
RMSF of CsgA
B-Factor loss in response to surface contact
Equilibration results of chiral models
fig. S1. Sequence diagram of curli subunit CsgA.
fig. S2. Tyrosine interactions with silica.
fig. S3. Energetic contributions summed by type on silica and maximum energetic repulsions with the silica surface.
fig. S4. RMSF by surface type for internal RMSF and translational RMSF.
fig. S5. Initial and final conformations of CsgA.
fig. S6. B-Factor decrease during adsorption.
fig. S7. Definition of clusters by residue ID.
fig. S8. Equilibration results of left- and right-handed CsgA.
REFERENCES AND NOTES

1. S. L. Hodson, C. M. Burke, A. P. Bissett, Biofouling of fish-cage netting: The efficacy of a silicone coating and the effect of netting colour. *Aquaculture* 184, 277–290 (2000).

2. W. G. Characklis, K. E. Cooksey, Biofilms and microbial fouling. *Adv. Appl. Microbiol.* 29, 93–138 (1983).

3. H.-C. Flemming, Biofouling in water systems—Cases, causes and countermeasures. *Appl. Microbiol. Biotechnol.* 59, 629–640 (2002).

4. R. M. Donlan, Biofilm formation: A clinically relevant microbiological process. *Clin. Infect. Dis.* 33, 1387–1392 (2001).

5. J. Garty, Influence of epithelial microorganisms on the surface temperature of building walls. *Can. J. Bot.* 68, 1349–1353 (1990).

6. K. Muffler, M. Lakatos, C. Schlegel, D. Striehi, S. Kuhre, R. Ulber, in *Productive Biofilms*, K. Muffler, R. Ulber, Eds. (Springer, 2014), pp. 123–161.

7. A. Gonzalez-Perez, K. M. Persson, Bioinspired materials for water purification. *Materials* 9, 447 (2016).

8. Z. Li, Y. Zhang, P. R. Le Duc, K. B. Gregory, Microbial electricity generation via microfluidic flow control. *Biotechnol. Bioeng.* 108, 2061–2069 (2011).

9. I. W. Sutherland, *The biofilm matrix—An immobilized but dynamic microbial environment*. Trends *Microbiol.* 9, 222–227 (2001).

10. B. Vu, M. Chen, R. J. Crawford, E. P. Ivanov, Bacterial extracellular polysaccharides involved in biofilm formation. *Molecules* 14, 2535–2554 (2009).

11. R. Duddy, D. L. Chopp, B. Moran, A two-dimensional continuum model of biofilm growth incorporating fluid flow and shear stress based detachment. *Biotechnol. Bioeng.* 103, 92–104 (2009).

12. J. N. Wilking, T. E. Angelini, A. Seminara, M. P. Brenner, D. A. Weitz, Biofilms as complex fluids. *MRS Bull.* 36, 385–391 (2011).

13. M. C. M. Van Loosdrecht, S. J. Heijnen, Biofilm bio-reactors for waste-water treatment. *Trends in Biotechnol.* 11, 117–121 (1993).

14. M. L. Evans, M. R. Chapman, Curli biogenesis: Order out of disorder. *Biochim. Biophys. Acta* 1843, 1551–1558 (2014).

15. M. R. Chapman, L. S. Robinson, S. J. Hultgren, *Escherichia coli’s how-to guide for forming amyloid*. *ASM News* 69, 121–126 (2003).

16. D. C. V. De Oliveira, A. Fernandez Jünger, R. Kaneno, M. G. Silva, J. P. Araújo Júnior, N. C. C. Silva, V. L. M. Rall, Ability of *Salmonella* spp. to produce biofilm is dependent on temperature and surface material. *Foodborne Pathog. Dis.* 11, 478–483 (2014).

17. M. S. Dueholm, S. B. Nielsen, K. L. Hein, P. Nislsen, M. Chapman, G. Christiansen, P. H. Nielsen, D. E. Otzen, Fibrillation of the major curli subunit CsgA under a wide range of conditions implies a robust design of aggregation. *Biochemistry* 50, 8281–8290 (2011).

18. E. K. Andersson, C. Bengtsson, L. M. Evans, E. Chorell, M. Sellstedt, A. E. G. Lindgren, D. A. Hufnagel, M. Bhattacharya, P. M. Tessier, E. Wittung-Stafshede, F. Almqvist, M. Bhattacharya, P. M. Tessier, E. Wittung-Stafshede, F. Almqvist, M. Chapman, G. Christiansen, P. H. Nielsen, D. E. Otzen, Fibrillation of the major curli subunit CsgA under a wide range of conditions implies a robust design of aggregation. *Biochemistry* 50, 8281–8290 (2011).

19. Y. Kai-Larsen, P. Lüthje, M. Chromise, V. Peters, X. Wang, A. Holm, L. Kádo-Hedlund, J. Johansson, M. R. Chapman, S. H. Jacobson, B. Agerberth, A. Brauner, Uropathogenic *Escherichia coli* medulates immune responses and its curli fibrin interacts with the antimicrobial peptide LL-37. *PLoS Pathog.* 6, e1001010 (2010).

20. C. Wu, Y. J. Lim, G. G. Fuller, L. Cegelski, Quantitative analysis of amyloid-integrated biofilms formed by uropathogenic *Escherichia coli* in the air-liquid interface. *Biophys. J.* 103, 464–471 (2012).

21. P. Goyal, P. V. Krasteva, N. Van Gerven, F. Gubellini, I. Van den Broeck, P. Goyal, P. V. Krasteva, N. Van Gerven, F. Gubellini, I. Van den Broeck, P. Goyal, P. V. Krasteva, N. Van Gerven, F. Gubellini, I. Van den Broeck, P. Goyal, P. V. Krasteva, N. Van Gerven, F. Gubellini, I. Van den Broeck, P. Goyal, P. V. Krasteva, N. Van Gerven, F. Gubellini, I. Van den Broeck, P. Goyal, P. V. Krasteva, N. Van Gerven, F. Gubellini, I. Van den Broeck, P. Goyal, P. V. Krasteva, N. Van Gerven, F. Gubellini, I. Van den Broeck, P. Goyal, P. V. Krasteva, N. Van Gerven, F. Gubellini, I. Van den Broeck, P. Goyal, P. V. Krasteva, N. Van Gerven, F. Gubellini, I. Van den Broeck.

22. T. Schubert, P. Yuan, M. Ahmed, M. Nagaraj, B.-J. van Rossouw, C. Ritter, Untangling a repetitive amyloid sequence: Correlating biofilm-derived and segmentally labeled curli fibrin by solid-state NMR spectroscopy. *Angew. Chem. Int. Ed.* 54, 14669–14672 (2015).

23. P. Sheemaker, R. K. Thurber, P. McPhie, F. Dyda, R. Tycko, R. B. Wickner, The functional curli amyloid is not based on in-register parallel β-sheet structure. *J. Biol. Chem.* 284, 25065–25076 (2009).

24. S. K. Collinson, J. M. R. Parker, R. S. Hodges, W. H. Kay, Structural predictions of AgfA, the insoluble fibrillar subunit of *Salmonella* thin aggregative fibril. *J. Mol. Biol.* 290, 741–756 (1999).

25. C. Zhong, T. Gurry, A. A. Cheng, J. Downey, Z. Deng, C. M. Stultz, T. K. Lu, Strong underwater adhesives made by self-assembling multi-protein nanofibers. *Nat. Nanotechnol.* 9, 858–866 (2014).

26. A. Y. Chen, Z. Deng, A. N. Billings, U. O. S. Seker, M. Y. Lu, R. J. Citronik, B. Zakeri, T. K. Lu, Synthesis and patterning of tunable multiscale materials with engineered cells. *Nat. Mater.* 13, 515–523 (2014).

27. P. O. Nguyen, Z. Botyanszki, P. K. R. Tay, N. S. Joshi, Programmable biofilm-based materials from engineered curli nanofibers. *Nat. Commun.* 5, 4945 (2014).
56. H. Heinz, Computational screening of biomolecular adsorption and self-assembly on nanoscale surfaces. J. Comput. Chem. 31, 1564–1568 (2010).

57. A. N. Camdem, S. A. Barr, R. J. Berry, Simulations of peptide-graphene interactions in explicit water. J. Phys. Chem. B 117, 10691–10697 (2013).

58. R. B. Pandey, Z. Kuang, B. L. Farmer, S. S. Kim, R. R. Naik, Stability of peptide (P1 and P2) binding to a graphene sheet via an all-atom to all-residue coarse-grained approach. Soft Matter 8, 9101–9109 (2012).

59. N. Dragneva, W. B. Floriano, D. Stauffer, R. C. Mawhinney, G. Fanchini, O. Rubel, Favorable adsorption of capped amino acids on graphene substrate driven by desolvation effect. J. Chem. Phys. 139, 174711 (2013).

60. P. E. Scopelliti, A. Borgonovo, M. Indrieri, L. Giorgetti, G. Bongiorno, R. Carbone, A. Podestà, P. Milani, The effect of surface nanometre-scale morphology on protein adsorption. PLoS ONE 5, e11862 (2010).

61. E. Gazit, A possible role for π-stacking in the self-assembly of amyloid fibrils. FEBS J. 16, 77–83 (2002).

62. G. B. McGaughhey, M. Gagné, A. K. Rappé, π-Stacking interactions alive and well in proteins. J. Biol. Chem. 273, 15458–15463 (1998).

63. P. A. Mirau, R. R. Naik, P. Gehring, Structure of peptides on metal oxide surfaces probed by NMR. J. Am. Chem. Soc. 133, 18243–18248 (2011).

64. C. Mücksch, H. M. Urbassek, Adsorption of BMP-2 on a hydrophobic graphite surface: A molecular dynamics study. Chem. Phys. Lett. 510, 252–256 (2011).

65. C. H. Yu, M. A. Norman, S. Q. Newton, D. M. Miller, B. J. Teppen, L. Schäfer, Molecular dynamics simulation of the adsorption of proteins on clay mineral surfaces. J. Mol. Struct. 556, 95–103 (2000).

66. T. Ragalauksas, M. Mickevicius, R. Budvytyte, G. Niaura, B. Carbonnier, G. Valincius, Adsorption of β-amyloid oligomers on octadecanethiol monolayers. J. Colloid Interface Sci. 425, 159–167 (2014).

67. F. Shewmaker, R. P. McGlinchey, R. B. Wickner, Structural insights into functional and pathological amyloid. J. Biol. Chem. 286, 16533–16540 (2011).

68. W. Humphrey, A. Dalke, K. Schulten, VMD: Visual molecular dynamics. J. Mol. Graph. 14, 33–38 (1996).

69. J. C. Phillips, R. Braun, W. Wang, J. Gumbart, E. Tajkhorshid, E. Villa, C. Chipot, R. D. Skeel, L. Kälé, K. Schulten, Scalable molecular dynamics with NAMD. J. Comput. Chem. 26, 1781–1802 (2005).

70. W. L. Jorgensen, J. Chandrasekhar, J. D. Madura, R. W. Impey, M. L. Klein, Comparison of simple potential functions for simulating liquid water. J. Chem. Phys. 79, 926–935 (1983).

71. A. D. MacKerell Jr., D. Bashford, M. Bellott, R. L. Dunbrack Jr., J. D. Evanseck, M. J. Field, S. Fischer, J. Gao, H. Guo, S. Ha, D. Joseph-McCarthy, L. Kuchnir, K. Kuczera, F. T. K. Lau, C. Mattos, S. Michnick, T. Ngo, D. T. Nguyen, B. Poothom, W. E. Reiher, B. Roux, M. Schlenkrich, J. C. Smith, R. Stote, J. Straub, M. Watanabe, J. Wróblewicz-Kuczera, D. Yin, M. Karplus, All-atom empirical potential for molecular modeling and dynamics studies of proteins. J. Phys. Chem. B 110, 3586–3616 (1998).

72. E. P. DeBenedictis, J. Liu, S. Keten, Adhesion mechanisms of curli subunit CsgA to abiotic surfaces. Sci. Adv. 2, e1600998 (2016).