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Molecular mechanism of extreme mechanostability in a pathogen adhesin

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Abstract

High resilience to mechanical stress is key when pathogens adhere to their target and initiate infection. Using atomic force microscopy-based single molecule force spectroscopy we explore the mechanical stability of the prototypical staphylococcal adhesin SdrG, which targets a short peptide from human fibrinogen β . Steered molecular dynamics simulations revealed and single-molecule force spectroscopy experiments confirmed the mechanism by which this complex withstands forces over 2 nN, a regime previously associated with the strength of a covalent bond. The target peptide, confined in a screw-like manner in the binding pocket of SdrG, distributes forces mainly towards the peptide backbone through an intricate hydrogen bond network. Thus, these adhesins can attach to their target with exceptionally resilient mechanostability, virtually independent of peptide side chains.

ONE SENTENCE SUMMARY

The > 2 nN mechanostability of a staphylococcal adhesin binding its human target is virtually independent of peptide side chains.

Gram-positive pathogenic bacteria have developed an arsenal of virulence factors specifically targeting and adhering to their host's proteins. Termed microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), they promote "adhesion, invasion and immune evasion" (1) (Fig. 1A). The prototypical adhesin is SD-repeat protein G (SdrG) from *Staphylococcus epidermidis*, the leading cause of medical device and implant related nosocomial infections (2). SdrG uses a key motif found in pathogenic *Staphylococci* the "Dock, Lock and Latch mechanism" (DLL) in which the host target, usually a peptide on the order of 15 residues, is first bound (dock), then buried (lock) between two Immunoglobulin-like fold (Ig) domains N2 and N3 (3). Finally, the target is snugly locked in place with a strand connecting N3 to N2 by β -strand complementation (latch, Fig. 1B) (4). The DLL mechanism has appeared in many homologous domains e.g. in *Staphylococcus aureus* with targets such as Keratin (5), complement system proteins (6), other chains of fibrinogen (7) and collagen (8). SdrG uses the DLL to target the N-terminus

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of the β -chain of human fibrinogen (Fg). The Fg sequence bound by SdrG is also the substrate of thrombin (Fg β , NEEGFFSARGHRPLD, thrombin cleavage between R and G). However, once bound by SdrG it can no longer be cut by thrombin, a step necessary for blood clotting and fibrin formation (9). Thrombin cleavage also releases fibrinopeptide B, which in turn recruits neutrophils. Additionally, the adhesin coats and thus camouflages the bacterium in host proteins. Combined, these MSCRAMM mechanisms allow *Staphylococci* to evade immune response, making them attractive targets for drug development, such as designing MSCRAMM inhibitors for anti-adhesion therapy (10, 11).

Here, we use the interplay between atomic force microscopy-based (AFM) single molecule force spectroscopy (SMFS) (12–14) and all-atom steered molecular dynamics (SMD) simulations to elucidate the mechanics of the SdrG:Fg β interaction with atomic resolution. Previous *in vivo* measurements using single-cell force spectroscopy of the SdrG fibrinogen interaction found adhesion forces on the order of 2 nN (15, 16), in addition comparable *in vivo* forces appeared in closely related adhesins (17, 18). In agreement with these results we measured rupture forces of more than 2 nN for a single SdrG:Fg β complex at force loading rates around 10^5 pN s $^{-1}$. This extreme stability is the highest among all non-covalent interactions by a large margin. SdrG:Fg β outperforms the current champion – the cohesindockerin type III interaction – by a factor of four (19), and Biotin-Streptavidin by more than an order of magnitude (20). It even rivals the strength of a covalent bond (21). Interestingly, the affinity between the peptide and SdrG is moderate, with a K_D around 300 nM (4). Accordingly, this system is adapted for strong mechanical attachment to its target, rather than high affinity. It was thus to be expected that these extreme SdrG:Fg β mechanics were governed by a special, currently unknown mechanism.

The Fg β wild-type (WT) peptide is located at the N-terminus of the mature Fg β chain. Thus, it can only be mechanically loaded from the C-Terminus (Fig. 1B). The SdrG N2 and N3 domains, responsible for binding the peptide (SdrG) are covalently anchored to the *S. epidermidis* cell wall by a C-terminal sortase motif. Hence, in the native, physiological configuration of the SdrG:Fg β complex, force is applied from the C-termini of both SdrG and Fg β . To mechanically probe this interaction, all surface anchoring onto AFM cantilever and surface was site-specific and covalent (Fig. 1C). To ensure unambiguous identification of single-molecule events in force-extension traces, a refolding molecular “fingerprint” (22) was cloned upstream of the peptide. Under physiologically relevant force loading from the C-terminus the complex withstood extremely high forces of up to 2500 pN *in vitro* (Fig. 1E, D) and even higher forces in corresponding SMD simulations (Fig. 1F, G), due to higher force loading rates *in silico* (23, 24) (see also Fig. S1-S3).

The force regime of 2 nN is typically associated with the stability of covalent bonds, raising the concern that our surface chemistry – not the complex – was breaking, most likely a Si-C bond in the aminosilane anchors used (21). As the cantilevers apexes have radii of around 10 nm, they can only present a few molecules. If the covalent attachment of SdrG to the tip was being mechanically cleaved, the SdrG coating on the apex of the tip would be left attached to the surface, resulting in a rapidly decreasing frequency of interactions over time. In contrast, a single cantilever remained active over thousands of interactions, indicating that covalent bonds in the surface functionalization largely sustained the high forces.

We were convinced that an alteration which lowered the unbinding force would be the key to deconstructing the mechanism of this exceptional mechanostability. The presence of the “bulky” hydrophobic amino acid side chains of two Phenylalanines (F) in Fgβ had been previously described as a “bulgy plug” (4). Buried behind the locking β-strand, it seemed conceptually and intuitively plausible that wiggling them through the narrow constriction created by the locking strand caused the high forces (Fig. 2 A, B, and Fig. S4).

The force dependence on the number of Fs was tested by addition of an F or by Alanine replacement. Four constructs were investigated: a Fgβ with three Phenylalanines (FgβF3), the WT Fgβ having 2 Fs, and mutants with one (FgβF1), or both (FgβF0), Fs replaced by Alanines. The F3 mutant had been shown to have higher affinity (K_D around 50 nM) for SdrG (4), whereas the affinity of the F1 mutant was lower compared to WT Fgβ, as the F’s hydrophobicity is important for initiating the DLL (25). All three mutants produced high forces around 2 nN (Fig. S5 A, B). A negative correlation of the most probable rupture force on the number of Fs was measureable, but only marginal (Fig. 2C). With reference to the Fgβ WT force, the most probable rupture force of the F0 mutant was only about 10% weaker than the WT. Multiple all-atom SMD simulations of all four systems, reproduced the miniscule correlation between the presence of bulky F side chains and the high-force regime (Fig. 2B). The F0 mutant was approximately 20% weaker than WT Fgβ. Thus, the bulky residues only contributed marginally to the high forces, whereas they had been established as crucial for initial binding (4).

As the bulky Phenylalanines in Fgβ were largely irrelevant for reaching high forces, we investigated minimizing the peptide. We employed QwikMD (26) to sequentially remove amino-acids from the N-terminus of Fgβ and tested their stability in SMD simulations. As expected, shortened peptides had lower unbinding forces. However, provided that the peptide was long enough to directly interact with SdrG’s locking strand, forces were still in the nN regime (Fig. 3A). Removing all residues contacting the locking strand up to Fgβ’s A13 eliminated clear complex rupture forces in the nN regime. Consequently, the minimal six residue peptide sequence in closest contact with the locking strand (FFSARG) was sufficient to both bind SdrG and withstand forces indistinguishable from WT Fgβ *in vitro* (Fig. 3B, S5C).

Provided a mutant could still bind SdrG, modifying the Fgβ peptide had only minor effects on mechanostability. Thus, we investigated the mechanical properties of SdrG. Previously, the presence and flexibility of the locking strand was shown to be crucial for the DLL mechanism and thus SdrG:Fgβ affinity (25). Locking strand deletion inhibits binding of Fgβ (4). In accordance with these results, a mutant SdrG(274-580) devoid of the locking strand failed to bind Fgβ *in vitro*. Still, the contribution of the locking strand to the mechanics was unclear. If the interaction between the N2 domain and the locking strand propagated force away from the complex, its truncation should significantly weaken rupture forces. A truncated SdrG(274-590) – which removed the locking strand’s C-terminal half of the “latch” region (Fig. S6) – still bound SdrG, yet its mechanostability was indistinguishable from the WT. Possible covalent isopeptide bonds (27, 28) between the locking strand and the N2 domain had been suggested to contribute to its stability. We could exclude this

hypothesis as cause of the unusually high mechanostability – as the SdrG truncation mutant removed D593 – a key amino acid required for a potential isopeptide bond (29).

As simulations and experiments strongly agreed, we were confident to explore mutants and setups created *in silico* that could not be realized *in vitro*. SMD became a gedankenexperiment to deconstruct the mechanism. It is important to emphasize that the strong agreement was provided in part by our enhanced sampling strategy (30). Performing many (at least 50 per system, over 2400 in total, overview in supplementary table S20) simulation replicas allowed the comparison of simulation and experiment within the same theoretical framework of the Bell-Evans (BE) and Dudko-Hummer- Szabo (DHS) models (24, 31, 32).

Simulations revealed the presence of strikingly frequent and persistent hydrogen bonds (H-bonds) between the Fg β peptide backbone and SdrG (Fig. 3D, F, Fig. S7, and Fig. S8). We investigated the contribution of the backbone H-bonds in SMD simulations by replacing Fg β with a peptide of purely glycines, which has no side chains. *In silico*, the rupture forces were merely 27 % weaker than the WT, comparable to the Fg β F0 mutant (Fig. 3E). Thus, we updated our initial hypothesis: reaching the regime of 2 nN was largely independent of Fg β 's side chains and mainly caused by SdrG interacting with the Fg β peptide backbone (Fig. S9, S10). Breaking the SdrG:Fg β complex in the native configuration requires all H-bonds to be broken in parallel: a cooperative shear geometry (see supplementary video S21).

Similar shear geometries appear in folds such as the muscle protein Titin-Ig. However, this protein disintegrates at lower forces around 200 pN (33), in stark contrast to SdrG's over 2000 pN. The shear geometry in Titin breaks as its backbone H-bonds have the freedom to move orthogonally to the force load, ultimately circumventing the shear geometry (34). In the SdrG:Fg β complex the peptide is snugly confined in the interface between N2 and N3 domain by the locking strand (Fig. S10, S11). The rigid and coiled (Fig. 3C, S12) alignment of the two interacting backbones neither bends nor buckles. Peptide movement orthogonal to the pulling force vector is not possible, so all H-bonds must be broken at once. The importance of this packed confinement was also demonstrated by analyzing the correlation-based dynamical network (35), which shows how force propagates through the system (Fig. S13) and how atom motion is clustered in communities (Fig. S14). These analyses revealed that force is propagated not directly by the latch strand, as demonstrated experimentally, but by neighboring strands, reducing the load over the H-bonds. Notably, the movement of Fg β peptide and both the N2 and N3 domain were highly correlated. To demonstrate the importance of the correct H-bond alignment, Fg β was tethered non-natively from its N-Terminus, effectively pulling orthogonally to the native force propagation. The non-native pulling of Fg β peaked at forces around 60 pN (Fig. 4A), over 40-fold smaller than the native configuration (Fig. S15). Simulations showed that this geometry is weaker, because the interactions between N2 and N3 are broken resulting in a loss of peptide confinement (see Fig. S16, S17, and supplementary video S22).

In a simplified model the DLL mechanism creates a deep and rigid binding pocket for the peptide, which is confined in a coiled geometry similar to a corkscrew in a cork (Fig. S12,

S18). If pulled upon, the load is dissipated cooperatively over all H-bonds that are radially pointed outwards of Fg β (Fig. 3G), causing the high mechanostability.

The importance of these H-bonds was confirmed in an exploratory SMD through removing coulomb interactions from parts of the peptide required for hydrogen bonding. Eliminating backbone H-bonds resulted in a significant reduction in rupture force *in silico* (Fig. 3E). Additionally, eliminating hydrogen bonds formed by the side chains of Fg β further reduced the forces, but only marginally – in agreement with the mechanism proposed (Fig. 3E). Still, the forces observed were only about 40% smaller than the WT. Furthermore, we tested turning off H-bonds of the all-Glycine peptide, which finally led to no detectable peak in the force profile. H-bonds with the peptide backbone were key to the mechanostability.

A pure glycine sequence, *i.e.* no side chains, showed high forces when bound to SdrG *in silico*. An analogous experiment was not possible, as such a sequence did not bind SdrG. The side chains such as the hydrophobic Phenylalanine residues were not essential for mechanostability but crucial for affinity. A homologous DLL motif adhesin, clumping factor B (ClfB) from *S. aureus*, had been found to promiscuously bind short sequences of extracellular matrix proteins. Among its targets is a C-terminal cytoskeletal Keratin peptide (K10, YGGGSSGGSSGGGH) (5). This unusually unremarkable target is essentially a flexible linker terminating in a charged residue. K10 contains no bulky, charged, or hydrophobic side chains, except for the C-terminal Histidine, secured by the locking strand in the complex structure. ClfB:K10 interactions also exceed the 2 nN mark, both *in vitro* and *in silico* (see Fig. 4B, C). More prominently than in SdrG, ClfB's mechanostability must be based on H-bonds to the K10 backbone, simply because it has no notable side chains. In last consequence, even a shortened K10 and pure GS sequence (GGGSSGGSSGGG) binds ClfB and reaches more than 2nN in force (Fig. S19). Moreover, the peptide β sheet is parallel to the locking strand, whereas the orientation is antiparallel in SdrG. Accordingly, it was natively tethered from its N-terminus, showing that nN stability is also possible for an inversely oriented peptide configuration. Finally, to generalize the mechanics we probed four additional homologs of SdrG and ClfB, all from *S. aureus*. SD repeat protein E (SdrE), clumping factor A (ClfA), bone sialoprotein binding protein (Bbp), and fibronectin binding protein A (FnBPA), had been crystallized with a known ligand bound (Fig. 4D) (36–39). Although most probable rupture forces varied up to 20 % depending on the adhesin, the overall forces were consistently in the 2 nN regime (Fig. 4E, F).

Side chain independent mechanics confer an invasive advantage to *Staphylococci*. No matter what sequence is targeted by their adhesins, invading pathogens using the DLL mechanism can adhere to their hosts even under the most demanding mechanical stress. One could speculate that this mechanism provides a flat fitness landscape. Adaption to a target will automatically yield extremely resilient mechanics, even if the sequence is mainly glycines and serines. The moderate bulk affinity of SdrG:Fg β allows for flexible unbinding and rebinding when no mechanical stress is applied. One can speculate that a high complex lifetime under force, which seems probable given the overall extreme mechanostability, is indicative of a very different unbinding pathway, when compared to the moderate lifetimes of spontaneous unbinding in bulk experiments (4). Thus, these differing pathways would

make a catch-bond behavior not surprising, considering such bonds have been found in bacterial adhesins with similar functions, albeit much lower mechanical strength (40, 41).

In conclusion, SdrG:FgB and its homologs are the mechanically strongest non-covalent protein-protein receptor-ligand interactions to date, rivaling a regime formerly exclusively associated with covalent bonds. The DLL mechanism creates a deep and rigid binding pocket confining the target in a stable geometry, that mainly relies on backbone H-bonds. Hence, the mechanostability of the complex only marginally depends on the target side chains and thus sequence – even if it is minimized to merely six amino acids. These adhesins are hyperstable protein handles suitable for mechanochemistry and able to unfold almost any protein. They may serve as templates to design even stronger ones – a non-covalent superglue (42). The mechanism proposed provides an atomistic understanding of why these adhesins can adhere to their hosts so resiliently, from which possible routes to inhibit it and impede *staphylococcal* adhesion may be derived.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. The SdrG:Fgβ complex withstands enormous forces *in vitro* and *in silico*.

(A) SdrG function, attached to the N-terminal peptide of the fibrinogen (purple) β chain (orange) adsorbed on a surface. This interaction prevents detachment of the bacterium by hydrodynamic forces. (B) Structure of the SdrG (blue):Fgβ (orange) complex. The locking strand (green) encloses the peptide in the binding pocket between the Ig-fold N2 (light blue) and N3 (dark blue) domain and a calcium (yellow) binding loop. The red arrows indicate the force applied to the molecular complex. (C) Experimental AFM setup including the ddFLN4 fingerprint domain (cyan). All constructs are covalently bound to the surface via Polyethyleneglycol (PEG) linkers and the ybbR-tag (yellow dots). In the native configuration, Fgβ and SdrG are force loaded from their respective C-termini. The AFM cantilever is retracted at constant velocity until the complex breaks. (D) Resulting force-extension trace in the native force propagation (blue), as it would occur at sites of *staphylococcal* adhesion. The distinctive fingerprint unfolding around 90 pN ddFLN4 (black arrow) featuring a substep was used to find specific interactions. It is followed by SdrG:Fgβ complex rupture, here at almost 2500 pN. (E) Dynamic force spectrum of the SdrG:Fgβ native geometry at cantilever retraction velocities 0.4 $\mu\text{m s}^{-1}$ (triangles, $N = 749$), 0.8 $\mu\text{m s}^{-1}$

(squares, $N = 696$), $1.6 \mu\text{m s}^{-1}$ (diamonds, $N = 758$), $3.2 \mu\text{m s}^{-1}$ (forward triangles, $N = 749$), $6.4 \mu\text{m s}^{-1}$ (circles, $N = 851$), with corresponding complex rupture force histograms for each velocity projected onto individual axes on the right. A Bell-Evans (BE) model fit (dotted line, $x = 0.051 \text{ nm}$, $k_{\text{off}}^0 = 9.2\text{E-}11 \text{ s}^{-1}$) through the most probable rupture force and force loading rate of each velocity (large open markers, with errors given as full-width at half maximum for each distribution) shows the expected force loading-rate dependency of the rupture force. (F) SMD force-extension trace (blue) in the native force propagation of SdrG:Fg β including experimental peptide linkers. The complex ruptured at almost 4000 pN, the extension is shorter than in the experimental trace, as there are no PEG spacers. The peak following the highest force peak corresponds to another metastable geometry after slipping of the Fg β peptide, that is below the resolution limit of our AFM. (G) The experimentally determined dynamic force spectrum from velocities of 0.4 to $6.4 \mu\text{m s}^{-1}$ for the native propagation from (E) is shown condensed as open circles. The dynamic force spectrum of steered MD simulations for velocities of ($25,000 \mu\text{m s}^{-1}$ to $12,500,000 \mu\text{m s}^{-1}$, triangle $N = 49$, square $N = 50$, diamond $N = 100$, forward triangle $N = 200$, pentagon $N = 147$, inverted triangle $N = 200$, respectively). Fits through SMD and experimental data, for BE model (gray, dotted line, $x = 0.047 \text{ nm}$, $k_{\text{off}}^0 = 1.0\text{E-}9 \text{ s}^{-1}$) and fit of a model by Dudko et. al. (DHS model, cusp potential $x = 0.12 \text{ nm}$, $k_{\text{off}}^0 = 6.1\text{E-}22 \text{ s}^{-1}$, $G^{++} = 78 \text{ k}_\text{B}T$, cyan dashed line and linear-cubic potential $x = 0.093 \text{ nm}$, $k_{\text{off}}^0 = 7.7\text{E-}18 \text{ s}^{-1}$, $G^{++} = 66 \text{ k}_\text{B}T$, brown dash-dotted line, both at $T = 300 \text{ K}$). *In vitro* and *in silico* data agree exceptionally well, although they are separated by six orders of magnitude in force loading rate and can be fit with a single model.

Fig. 2. Phenylalanine side chains only marginally influence SdrG:Fgβ mechanostability. (A) Sketch of the “bulgy plug” hypothesis. The bulky phenylalanine side chains (gray) of Fgβ (orange) are blocked by the locking strand (green). (B) Crystal structure showing the bulky phenylalanine sidechains in van der Waals representation (gray spheres) of Fgβ (orange). They have to wiggle through a narrow constriction (cyan surface). (C) Dependence of complex rupture force on the presence of phenylalanines, if replaced by alanines. Most probable rupture forces (absolute values in bar graphs) are compared relative to WT Fgβ. Either recorded experimentally with a single cantilever retracted at $1.6 \mu\text{m s}^{-1}$ or corresponding results for SMD simulations at $250,000 \mu\text{m s}^{-1}$. Adding one F (FgβF3 mutant) slightly increases forces. Yet, both results show a trend of weak dependence of rupture force on the presence of phenylalanines. Even when removing all bulky side chains (FgβF0 mutant) experimental rupture forces drop no more than 10% compared to WT Fgβ, *in silico* no more than 20%. The “bulgy plug” only marginally contributes, hinting that another mechanism must be responsible for the high forces.

Fig. 3. Backbone H-bonds are deciding factors in the high mechanostability of SdrG:Fgβ and a minimized peptide.

(A) Fgβ peptide truncations from the N-terminus *in silico*. By removing amino acids the forces drop (relative to the WT) with the most significant drop when removing the sequence FSAR, leading to FFSARG as the minimum peptide. (B) Rupture forces for SdrG binding to WT Fgβ (green, continuous line, N = 437), and the six-residue minimized peptide FFSARG (orange, dash-dotted line, here shown with surrounding amino acids in gray, N = 471). Strikingly, there is hardly any difference between WT Fgβ and the minimized peptide. (C) Rupture force histograms comparing the wildtype Fgβ:SdrG interaction (green, continuous line, N = 463), and the SdrG mutant with the truncated latch region (red, dashed line, N = 131). WT and mutant are virtually indistinguishable (no significant difference in Kolmogorov–Smirnov test, *in vitro* $p = 0.29$, *in silico* $p = 0.88$). Corresponding SMD results WT N = 100, mutant N = 50) are shown as inset. (D) Relative prevalence (bar graphs, precise values in Fig. S7) of H-bonds between SdrG domains, the locking strand and the WT

Fg β peptide (also available for F3, F1, F0, and all-Glycine mutants in Fig. S8). The locking strand connects to nearly every Fg β residue. (E) Rupture forces from exploratory simulations for SdrG and Fg β WT (green, continuous line, N = 100), a replacement of each Fg β residue with glycine (blue, dash-dotted line, N = 100), Fg β F3 peptide without coulomb interactions, and subsequently H-bonds, on its backbone (orange, dashed line, N = 47), Fg β F3 devoid of all coulomb interactions (red, dotted line, N = 48). Backbone H-bonds in the Fg β confinement allow even a pure glycine sequence to withstand high force. (F) H-bond (purple) contacts respective to the backbone of Fg β (orange) and locking strand (green) confined by SdrG (white surface) from simulations in a force loaded state. The minimum peptide sequence is highlighted in the red box (G) Radial distribution of backbone H-bonds between locking strand (green) caused by the screw-like winding of the Fg β sheet (orange). Peptide backbones are shown as sticks.

Fig. 4. A non-native SdrG:Fg β force loading shows weak forces, a homologous domain ClfB reaches 2 nN stability binding a mainly glycine-serine peptide and SdrG homologs consistently exceed 2 nN binding to their ligands.

(A) Dynamic force spectrum of the SdrG:Fg β non-native configuration, see inset with purple arrow, breaking around 60 pN as opposed to > 2 nN for the native case (for SMD results see Fig. 15-17 and video S22). Cantilever retraction velocities were varied: $0.4 \mu\text{m s}^{-1}$ (triangles, $N = 511$), $0.8 \mu\text{m s}^{-1}$ (squares, $N = 564$), $1.6 \mu\text{m s}^{-1}$ (diamonds, $N = 487$), $3.2 \mu\text{m s}^{-1}$ (forward triangles, $N = 395$), $6.4 \mu\text{m s}^{-1}$ (circles, $N = 471$) with corresponding complex rupture force histograms projected on the right. A BE model fit (dashed line) through the most probable rupture force and force loading rate of each velocity (large open markers) shows the expected force loading-rate dependency of the rupture force $x = 0.46 \text{ nm}$, $k_{\text{off}}^0 = 0.39 \text{ s}^{-1}$. (B) ClfB (blues):K10 (orange) complex including the locking strand (green) and H-bonding (purple) amino acids shown as sticks. Notably, the latch region was not crystallized and needed to be modeled from a homolog. The native pulling configuration is indicated with an arrow, compared to Fg μ the peptide is oriented inversely in the binding pocket. (C) Rupture force histogram and fit for ClfB:K10 at a velocity of $0.8 \mu\text{m s}^{-1}$ (green, dashed line, $N = 1035$) peaking around 2.3 nN. Simulation data ($N = 50$) confirming the force regime are shown as inset. (D) Homologous systems employing the DLL mechanism, all from *S. aureus* (N2 and N3 domains in blue, target peptides in orange) SdrE, Bbp, FnBPA, and ClfA (E) Comparison of absolute mechanostability of all homologous systems

as well as SdrG and ClfB with a single force probe. The cantilever is modified with five different peptides tethered in their native force loading geometry, respectively: from the C-terminus of Complement Factor H (CFH), Fga chain (Fga), and Fgβ, tethered from the N-terminus are sequences from Dermokine (DK), and Fgγ chain (Fgγ). This selection is presented to all adhesins, which are known to bind at least one of them, spatially separated on a surface. One cannot exclude that one adhesin may bind more than one peptide target. (F) Resulting relative stabilities of the complexes for SdrE (red, dashed line, N = 680), ClfB (orange, dash-dotted line, N = 605), ClfA (cyan, dashed line, N = 2292), Bbp (purple, dot-dot-dashed line, N = 319), SdrG (green, continuous line, N = 478), FnBPA (blue, dash-dash-dotted line, N = 2483). SdrG is not the strongest system at a retraction velocity of $1.6 \mu\text{m s}^{-1}$. In accordance with the largely side-chain independent mechanics proposed for SdrG and ClfB, every DLL adhesin withstands forces exceeding 2 nN.