

BIOPHYSICS

Ultrastrong *Staphylococcus aureus* adhesion to human skin: Calcium as a key regulator of noncovalent interactions

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Calcium is a critical regulator of *Staphylococcus aureus* skin adhesion, stabilizing one of the strongest noncovalent biomolecular interactions ever recorded. Using in vitro and in silico single-molecule force spectroscopy, we demonstrate that calcium ions (Ca^{2+}) are essential for the ultrastrong binding between the serine-aspartate repeat protein D (SdrD) adhesion and the human skin protein desmoglein-1 (DSG-1), withstanding forces exceeding 2 nanonewtons. Ca^{2+} ions stabilize both the SdrD complex and the mechanically robust SdrD B-domains, which exhibit unprecedented folding strength. In the context of atopic dermatitis (AD), disrupted calcium gradients amplify SdrD interactions, which could potentially intensify *S. aureus* virulence. Furthermore, abnormal DSG-1 distribution on AD-affected skin enhances bacterial adhesion. These findings provide crucial insights into the calcium-dependent regulation of bacterial adhesion and folding, suggesting possible therapeutic targets to combat *S. aureus* infections.

INTRODUCTION

The Gram-positive bacterium *Staphylococcus aureus* is a commensal organism found in ~20% of healthy adults (1–3). It is also an opportunistic pathogen capable of causing a broad spectrum of infections, ranging from superficial skin lesions to severe, life-threatening conditions such as endocarditis and septicemia. *S. aureus* is also known to exacerbate symptoms of atopic dermatitis (AD; eczema) (4–7), and its presence has been demonstrated to correlate with disease severity (8–11). The rising incidence of *S. aureus* infections is driven by increased colonization rates, a higher prevalence of immunosuppressive conditions, the widespread use of surgical implants, and the expansion of antibiotic resistance (12, 13). The emergence of methicillin-resistant *S. aureus* strains has further exacerbated treatment challenges by expanding resistance to multiple antibiotics (14, 15). Despite extensive research efforts, no vaccine against *S. aureus* has been approved to date (15, 16).

Skin colonization by *S. aureus* begins with its attachment to the stratum corneum (SC), the outermost layer of the epidermis (17). The SC consists of corneocytes, which are flattened dead cells filled with keratin and surrounded by a cornified envelope, in contrast to the living keratinocytes found in deeper layers. Corneocytes are connected by corneodesmosomes, primarily composed of desmoglein-1 (DSG-1), corneodesmosin (CDSN), and desmocollin-1. These proteins are localized only at the periphery of the cells (18), suggesting that their degradation at the corneocyte center could provide space for the

stable maintenance of intercellular lipids (19). DSG-1 is expressed on the surface of both corneocytes and keratinocytes. This cadherin-type cell adhesion protein has a calcium-dependent extracellular (EC) region with four cadherin repeats, an anchor domain, a transmembrane region, and a cytoplasmic domain (see fig. S1) (20). *S. aureus* exfoliative toxins cleave the homophilic interaction site of DSG-1, causing cell detachment (21) and compromising the SC barrier function (19). Mutations in DSG-1 are linked to dermatitis, multiple allergies, and metabolic wasting (22), highlighting DSG-1's essential role in maintaining skin integrity and its involvement in disease.

S. aureus adheres to corneocytes using cell wall-anchored surface proteins, including fibronectin-binding proteins A (FnBPA) and B (FnBPB), clumping factor B (ClfB), and iron-regulated surface determinant A (IsdA) (6, 23, 24). These adhesins form mechanostable bonds (25, 26) with target ligands such as keratin, loricrin, and CDSN. ClfB mediates *S. aureus* adhesion to corneocytes from patients with AD by binding to loricrin and cytokeratin via the dock, lock, and latch (DLL) mechanism (27, 28). Both FnBPB and ClfB interact with the N-terminal region of CDSN on AD corneocytes. An important challenge is to identify other adhesins that may play roles in the colonization of skin, their target ligands, and their binding mechanisms. The serine-aspartate repeat protein D (SdrD) was identified as a potential partner for DSG-1, which has been shown to promote *S. aureus* adhesion to human keratinocytes (29) and desquamated nasal epithelial cells (30), although its mechanisms remain unknown.

SdrD is part of the Sdr family of *S. aureus* surface proteins, characterized by an N-terminal signal peptide, followed by an A region composed of N1, N2, and N3 subdomains, variable numbers of B-repeats (110 to 113 residues), a Ser-Asp repeat (R) domain, and an Leu-Pro-X-Thr-Gly (LPXTG) cell wall-anchoring motif (see Fig. 1A and fig. S1) (31). The B-repeats of SdrD form a rigid rod-like structure, proposed to function as shock absorbers that project the A-domain away from the cell surface, with Ca^{2+} required for proper folding and structural integrity (see fig. S1) (25, 32, 33).

The remarkable architecture of *S. aureus* adhesins allows them to form mechanical bonds that are not only highly specific but also

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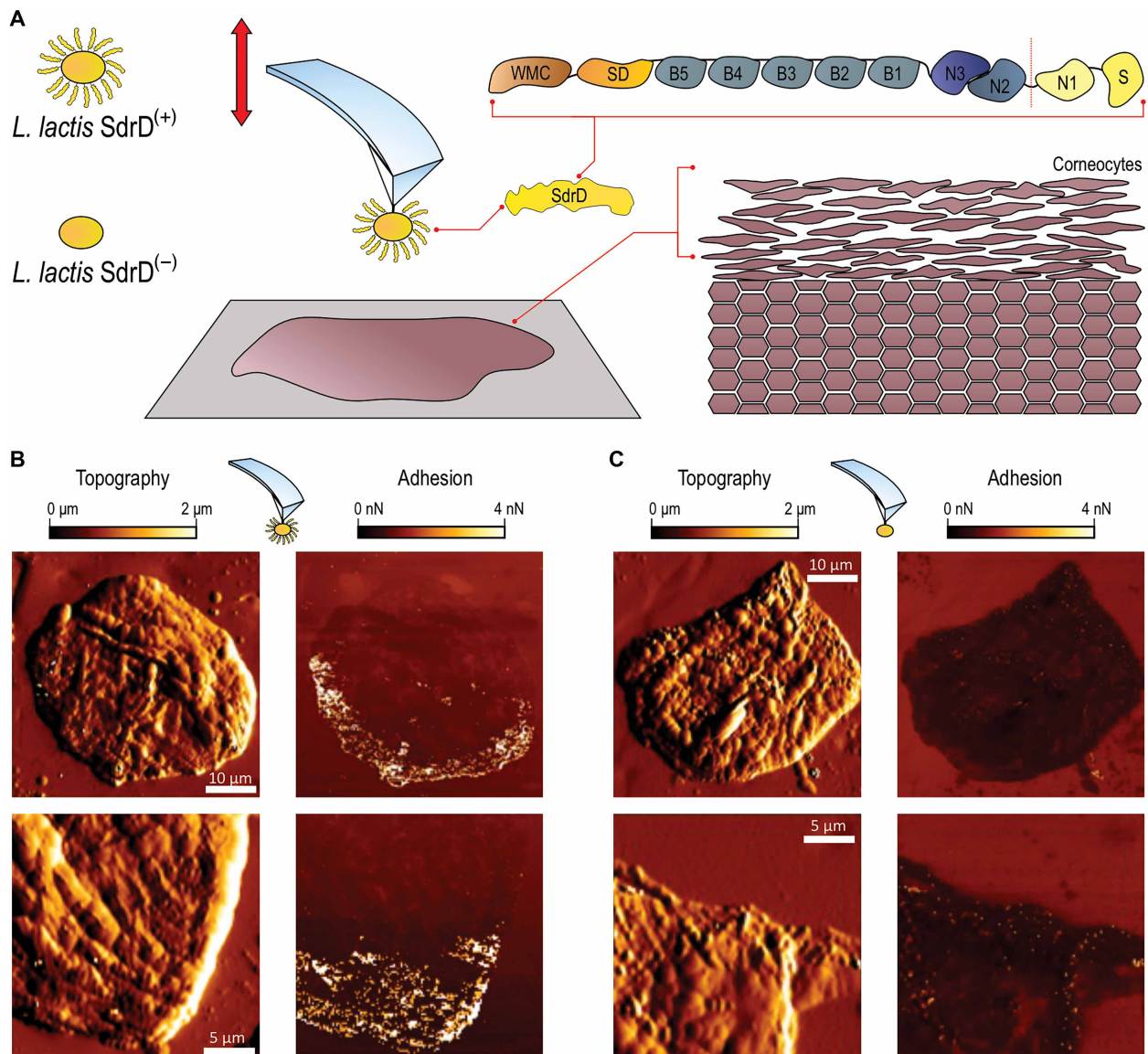


Fig. 1. SdrD mediates bacterial adherence to healthy corneocytes. (A) Schematic illustration of SCFS experiments where we mapped the molecular interactions between single *L. lactis* SdrD⁽⁺⁾ and *L. lactis* SdrD⁽⁻⁾ bacterial probes and corneocytes. SdrD different functional domains are described on an insert: WMC (Wall- and membrane-spanning domains); SD (serine-aspartate repeats); B-repeated regions; the A region comprising N1, N2, and N3 domains; and the S (signal peptide), removed posttranslationally. (B and C) Representative topographic images of the structure and adhesion of corneocytes recorded with *L. lactis* SdrD⁽⁺⁾ and *L. lactis* SdrD⁽⁻⁾, respectively. Adhesion featuring numerous strong rupture events is shown as bright pixels. One of the experiments is represented, over an $n = 3$ cells.

extremely resilient under force (34). Such mechanostable interactions are crucial for pathogen survival in dynamic environments like the skin or bloodstream, where shear forces constantly challenge adhesion. Several staphylococcal adhesins, including ClfA, ClfB, and the serine-aspartate repeat protein G (SdrG), form some of the strongest noncovalent protein-protein bonds known to date, with rupture forces exceeding 2 nN (35). These interactions are characteristic examples of catch bonds, whose lifetimes increase under mechanical load, thereby reinforcing bacterial attachment during colonization (36). Mechanostable bonds are not limited to bacterial systems. They are also prevalent in viral infections (37–40), where force-dependent interactions can regulate viral entry and immune evasion, and are increasingly recognized in the context of genetic diseases involving

mechanically sensitive protein complexes (41, 42). Beyond their roles in pathogenesis, these force-enhanced interactions have been widely adopted in biotechnology. Applications range from the well-known streptavidin:biotin pair (43, 44), valued for its extreme stability, to engineered therapeutic complexes such as PD-L1:affibody (45), which rely on tethering-specific mechanical resilience for improved performance.

In this study, we used single-cell force spectroscopy (SCFS) to investigate the binding of SdrD to healthy and AD corneocytes, focusing on the roles of DSG-1 and calcium in the adhesion mechanism. Our findings reveal that SdrD forms exceptionally mechanostable bonds with corneocytes, particularly DSG-1, enduring forces greater than 2 nN. Notably, the adhesion frequency was clearly higher in

AD corneocytes compared to healthy cells. By combining in vitro and in silico single-molecule force spectroscopy (SMFS), we identified the SdrD binding site and demonstrated that calcium stabilizes the complex by reinforcing the adjacent cadherin domains. The extraordinary hyperstability of the SdrD complex, which represents the strongest molecular complex known to date, is explained by a highly connected network of interactions, where the E-cadherin domain [extracellular domain 4 (EC4)] bridges key interface connections, further rigidifying the complex. These findings suggest that the DLL mechanism in this system is enhanced by the interplay between DSG-1 cadherins and the A-domain of SdrD.

RESULTS

Characterizing SdrD-mediated adhesion to corneocytes

To test whether SdrD promotes bacterial adhesion to corneocytes, we first mapped the molecular interactions between single bacterial probes and corneocytes from the SC of healthy subjects using SCFS in the quantitative imaging (QI) mode (46–48). In this method, a *Lactococcus lactis* bacterium, as a model system, is bound to the cantilever of an atomic force microscope, which is then moved over a surface containing the corneocytes. In our study, *L. lactis* bacteria expressing SdrD on their surface, SdrD⁽⁺⁾, and bacteria lacking this protein, SdrD⁽⁻⁾, were used, as shown in Fig. 1A. Adherence assays confirmed surface expression of SdrD in *L. lactis* SdrD⁽⁺⁾ (fig. S2). Representative correlated images of the topography and adhesion of corneocytes recorded with *L. lactis* SdrD⁽⁺⁾ bacterial probes are shown in Fig. 1B. Topographic images revealed fine structural details of the skin cell surfaces despite the large probe size. Notably, adhesion images featured numerous strong adhesion events (bright pixels), primarily localized on the edges of the corneocytes. These interactions were abolished when using *L. lactis* SdrD⁽⁻⁾ probes (Fig. 1C), indicating that they are exclusively mediated by SdrD.

To further characterize the interaction between SdrD and corneocytes, SCFS in the force-volume (FV) imaging mode was used. Figure 2 (A and C) and fig. S3 show a remarkable mechanostable adhesion between *L. lactis* SdrD⁽⁺⁾ and corneocytes, with a high adhesion frequency, while *L. lactis* SdrD⁽⁻⁾ did not exhibit any mechanostable events (Fig. 2, B and C, and fig. S4). For *L. lactis* SdrD⁽⁺⁾, ~70% of the force curves featured adhesive events, whereas these events were largely absent in *L. lactis* SdrD⁽⁻⁾ (Fig. 2C), underscoring the role of SdrD in the observed interactions. Additionally, SdrD-dependent adhesion forces were notably strong, with a sharp distribution centered at 2336 ± 263 pN (means $\pm$ SD), estimated from 2721 adhesion force profiles from nine different bacterium-corneocyte pairs (Fig. 2A and fig. S3). We propose that the 2.3-nN forces correspond to the rupture of single SdrD-ligand complexes, as similar narrow distributions of high forces were consistently observed in all cell pairs. Additionally, a less frequent population of unbinding forces peaked at 4135 ± 340 pN was observed, likely due to the rupture of two bonds in parallel (Fig. 2A and fig. S3). These bonds always ruptured at the same distance, 272 ± 62 nm, indicating parallel rather than serial bond ruptures. The binding strength of a single SdrD:corneocyte pair is comparable to that of the prototypical DLL interaction between SdrG and fibrinogen- β (Fg β) (35), suggesting that SdrD-ligand binding may proceed through a similar DLL mechanism.

Among the constituents of corneodesmosomes, SdrD has been shown to be able to bind DSG-1 (29). Binding events were mostly

distributed on the periphery of healthy corneodesmosomes, which have surfaces coated with DSG-1 proteins. Computational modeling and bioinformatics analyses were used to characterize the interaction between DSG-1 and SdrD. The first step was to predict the tridimensional structures of the outer membrane domains of DSG-1, which comprise four EC domains (Fig. 2D and fig. S1). Using this strategy, we identified two loop regions on DSG-1 as candidates for binding at the N2N3 interface of the SdrD A-domain. Both regions were primarily unstructured. Region 1, distal from the cell membrane at the N-terminal region of DSG-1, was predicted by bioinformatic analysis to form a β sheet with SdrD, resembling other adhesion structures (49–53). Region 2, proximal to the membrane, between the E-cadherin EC4 and the transmembrane region, did not show a stable secondary structure formation with SdrD (see Fig. 2E). Examining the crystal structure of the unbound (apo) form of SdrD [Protein Data Bank (PDB) ID: 4JE0] showed a high structural and sequence similarity in the peptide-binding area to that of ClfB (PDB ID: 4F27). We then aligned the protein sequence of Region 2 with the possible targets of ClfB found in the PDB (53). Our analysis also considered the Fg β complex with SdrG (PDB ID: 1R17) (49). Notably, one of the bulky phenylalanine (Phe) residues from Fg β aligns with Phe⁴⁸⁶ on DSG-1, a residue crucial for maintaining the integrity of the SdrG:Fg β complex (35). Within these two potential target regions, we selected two peptide sequences, here named p_{DIST} and p_{PROX} (Fig. 2E).

To confirm DSG-1 as the binding target of SdrD, we conducted SMFS using atomic force microscopy (AFM) tips modified with purified, full-length DSG-1 (Fig. 2F) to examine the binding affinity and mechanostability of the SdrD on living bacteria. The SMFS experiments, shown in Fig. 2G and fig. S5, revealed strong adhesion forces for *L. lactis* SdrD⁽⁺⁾, similar to those obtained with full corneocytes, at 2076 ± 248 pN, from 1229 adhesion force profiles (seven different cells). In contrast, *L. lactis* SdrD⁽⁻⁾ showed no mechanostable events (Fig. 2H and fig. S6). These results confirm DSG-1 as the SdrD target, which is also demonstrated by a drop in binding probability from 49% for SdrD⁽⁺⁾ to 6% for SdrD⁽⁻⁾ (Fig. 2I). Again, the high-force populations were comparable to the prototypical DLL interaction between SdrG and Fg β (35, 49, 54, 55). Unlike SCFS experiments, we did not observe higher forces associated with multiple parallel interactions, consistent with the lower density of interacting molecules on SMFS tips compared to SCFS bacterial probes. The rupture lengths were larger with DSG-1 than with corneocytes, averaging 431 ± 104 nm (Fig. 2, A and G, and fig. S5). This difference can be attributed to the transmembrane nature of DSG-1: While SMFS examines the full-length protein (1049 amino acids), SCFS focuses solely on its EC region (548 amino acids). The rupture length profile suggests that p_{PROX} is the likely binding target. The similarity in strong binding forces measured for SdrD binding to DSG-1 and to healthy corneocytes strongly suggests that the binding partners interact in situ. This is further supported by the structure of DSG-1, whose interacting region is located in the A-domain and likely involves a DLL mechanism, as revealed on corneocytes.

The SMFS experiments demonstrate that DSG-1 is the target of SdrD. While these experiments provided strong indications, particularly through the rupture length profiles, they could not definitively confirm whether p_{PROX} or p_{DIST} of DSG-1 is the specific binding region. To elucidate which region SdrD binds to, we conducted extensive computational experiments using an in silico SMFS approach (56). First, using MODELLER (57), we built all-atom three-dimensional (3D) models of the DSG-1 peptides p_{DIST} and

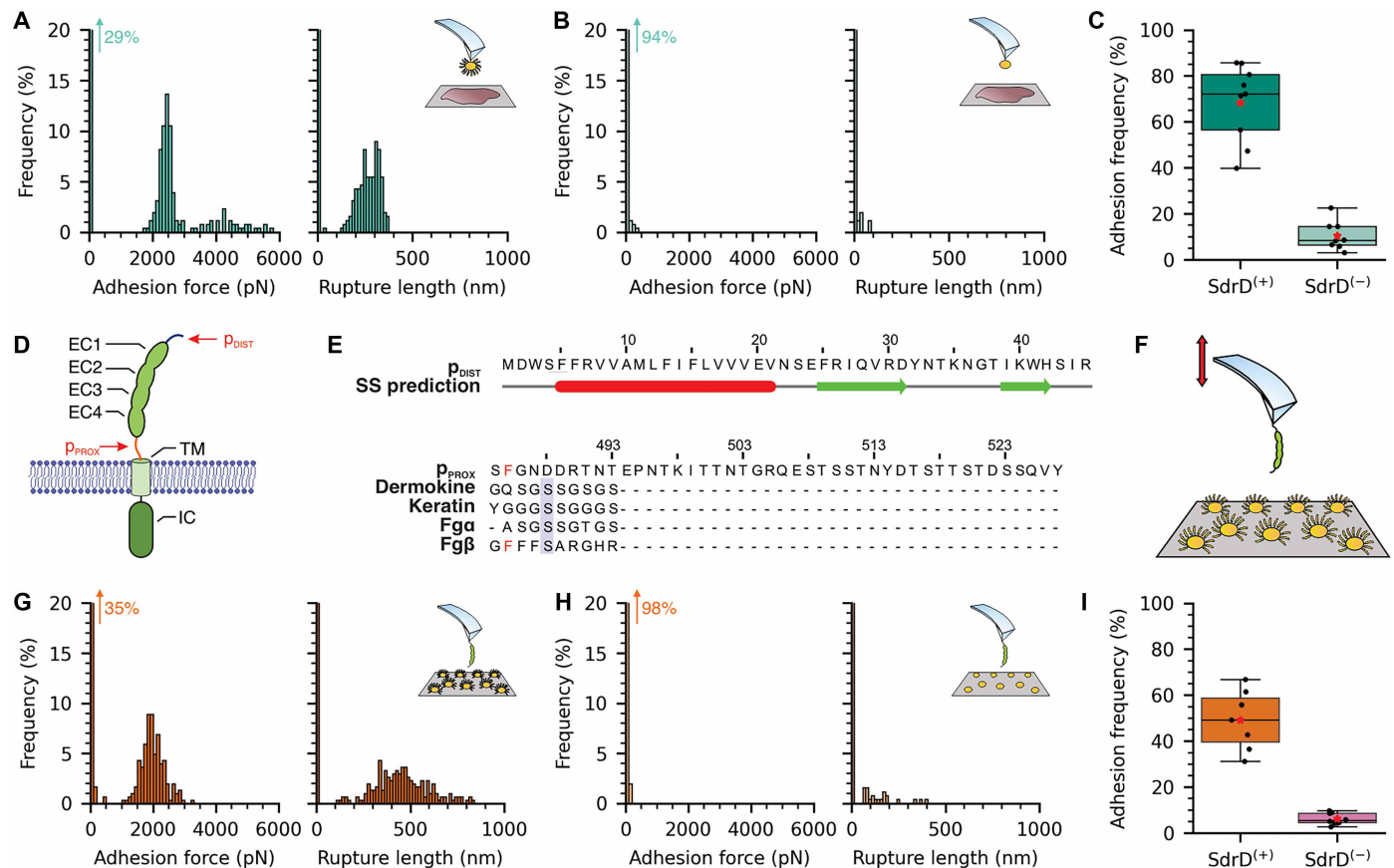


Fig. 2. DSG-1 is the target ligand of SdrD. (A) *L. lactis* SdrD⁽⁺⁾-dependent adhesion rupture forces were extremely strong, with a sharp distribution centered at 2336 pN. Rupture lengths are around 265 nm, consistent with the length of the adhesin. A representative histogram is shown ($n = 256$). (B) No adhesion rupture events are observed for *L. lactis* SdrD⁽⁻⁾. A representative histogram is shown ($n = 256$). (C) Adhesion frequencies estimated over the SMFS experiments for *L. lactis* SdrD⁽⁺⁾ and *L. lactis* SdrD⁽⁻⁾. (D) Schematic view for the DSG-1 protein, highlighting its four EC domains, the transmembrane (TM) region and the intracellular (IC) domain. Red arrows represent two unfolded regions that could be the potential binding sites for SdrD. (E) Bioinformatics analysis on two peptides located near EC1 and EC4, here called p_{DIST} and p_{PROX}, respectively. p_{DIST} sequence has propensity to form β strands, while p_{PROX} sequence does not; nevertheless, alignment with other peptides cocrystallized with ClfB, dermokine (PDB ID: 4F20), keratin (PDB ID: 4F1Z), and fibrinopeptide- α (PDB ID: 4F27), reveals a potential region near the transmembrane domain of DSG-1. In addition, we observe a conservation of a Phe residue present at fibrinogen- β (Fg β) (PDB ID: 1R17) that plays a key role for SdrD:Fg β adhesion. (F) Schematic representation for the SMFS experiment to investigate the binding of SdrD to DSG-1 by recording the force curves between *L. lactis* SdrD⁽⁺⁾ and *L. lactis* SdrD⁽⁻⁾ and atomic force microscopy (AFM) tips functionalized with DSG-1. (G) Similar to the values obtained with the full corneocytes, *L. lactis* SdrD⁽⁺⁾ adhesion rupture forces were very strong, around 2076 pN. A representative histogram is shown ($n = 304$). (H) No adhesion events were observed for the construct *L. lactis* SdrD⁽⁻⁾. A representative histogram is shown ($n = 256$). (I) Adhesion frequencies estimated over the SMFS experiments for *L. lactis* SdrD⁽⁺⁾ and *L. lactis* SdrD⁽⁻⁾.

p_{PROX} in complex with SdrD based on the solved apo structure of SdrD (33) and the crystallographic structure of SdrG complexed with Fg β (Fig. 3, A and B) (49). Specific to p_{PROX}, we incorporated the fourth EC E-cadherin domain (EC4) of DSG-1 into the model to assess its influence on the mechanostability of the complex, as this domain was found to be near the potential binding region (Fig. 3B). Using graphic processing units (GPU)-accelerated NAMD3 (58) through its QwikMD interface (59), we performed classical all-atom molecular dynamics (MD) simulations on explicitly solvated SdrD:-DSG-1 models. The binding free energy of p_{DIST} and p_{PROX} to SdrD was estimated using the molecular mechanics with generalized Born (GB) and surface area solvation (MM-GBSA) (Fig. 3C) (60, 61). The results show that p_{PROX} has a stronger binding affinity than p_{DIST}, with a difference of 5 kcal/mol.

We then performed all-atom steered MD (aa-SMD) simulations to determine the rupture forces for the SdrD complexes with both

p_{DIST} and p_{PROX}. The distribution of these forces, following the Bell-Evans (BE) fit, is presented in Fig. 3D. SdrD:p_{DIST} rupture forces showed a sharp distribution centered at 1360 ± 56 pN, while SdrD:-p_{PROX} rupture forces displayed a broader distribution centered at 3321 ± 56 pN. These data indicate that the SdrD:p_{PROX} complex exhibits greater mechanostability compared to the SdrD:p_{DIST} complex. The rupture forces for SdrD:p_{DIST} were low for adhesins (34, 35, 62). It has been previously hypothesized, through a combination of in silico and in vitro SMFS, that any peptide binding an adhesin via a DLL mechanism would exhibit nanonewton mechanostability at AFM-level force loading rates (35). However, due to limitations in computational methods, in silico force loading rates are much higher, resulting in higher rupture forces (35). Therefore, the ~ 1.4 -nN rupture forces measured by in silico SMFS suggest that rupture forces would be a fraction of nanonewton if tested with in vitro SMFS experiments. This result suggests that only p_{PROX} could account for the

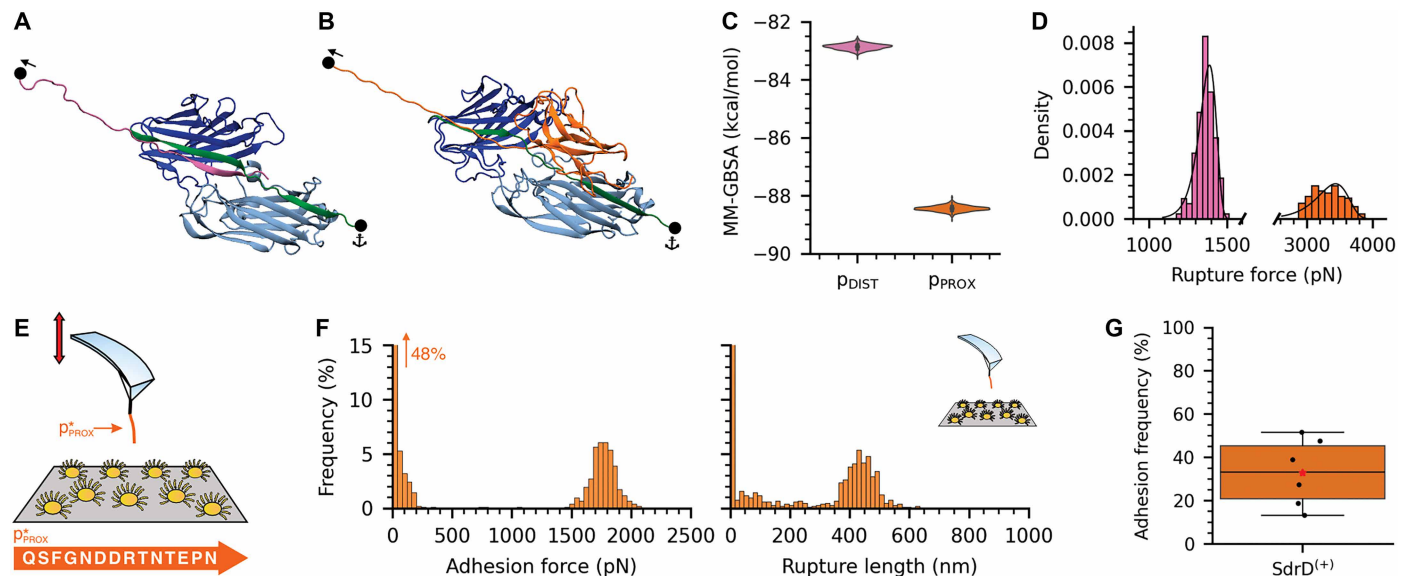


Fig. 3. SdrD binding site is predicted in silico and validated in vitro. (A and B) Three-dimensional (3D) structures for SdrD:DSG-1 complex with p_{DIST} or p_{PROX} at the interface, respectively. SdrD A-domain, represented by N2 and N3 domains are colored in dark and light blue, respectively, while p_{DIST} is colored in pink and p_{PROX} is colored in light orange. Anchor and pulling points used in the steered molecular dynamics (SMD) simulations are also indicated. Due to the physical proximity between p_{PROX} and EC4, the latter was included on SdrD:DSG-1 model, colored in bright orange. (C) Free energy of binding between SdrD and p_{DIST} or p_{PROX}, estimated from in-equilibrium MD simulations. (D) Adhesion rupture forces observed during SMD simulations for SdrD:p_{DIST} and SdrD:p_{PROX} complexes. Values observed for SdrD:p_{PROX} are clearly higher and pinpoint p_{PROX} as the probable binding site for SdrD. $n = 128$ and $n = 126$ for p_{DIST} and p_{PROX}, respectively. Pulling speed, 5.0×10^{-4} nm/ps. (E) Schematic view of the *L. lactis* SdrD⁽⁺⁾:p_{PROX} SMFS experiment; the sequence of p_{PROX} peptide, based on p_{PROX}, is shown. (F) Adhesion rupture forces between *L. lactis* SdrD⁽⁺⁾ and p_{PROX}. Strong rupture forces were detected around 1808 pN with rupture lengths around 400 nm ($n = 1024$). Pulling speed, $1 \mu\text{m s}^{-1}$. (G) Adhesion frequencies estimated over the SMFS experiments for *L. lactis* SdrD⁽⁺⁾:p_{PROX}. The boxplot shows data from $n = 6$ cells; red star indicates the mean value. Events with no adhesion were not considered on the boxplot.

high-force regimes observed in the AFM experiments presented in Fig. 2G and fig. S5. Additionally, the relatively low rupture force observed for SdrD:p_{DIST} may indicate a modified version of the traditional DLL mechanism. A visual inspection of our simulations revealed that the EC4 domain likely contributes to the high mechanostability of the SdrD:p_{PROX} interface. Also, the binding configuration of p_{PROX} and the high rupture forces observed are consistent with those seen for SdrG (36, 55, 63).

To further validate our in silico predictions, we performed SMFS experiments using a peptide construct derived from p_{PROX}, consisting of a shorter sequence encompassing only the main predicted binding interface. This minimal construct is hereafter referred to as p_{PROX}^{*} (Fig. 3E). Consistent with the results shown in Fig. 2G for the full-length DSG-1 construct, binding of p_{PROX}^{*} to SdrD exhibits nanonewton-range mechanostability, with rupture forces displaying a sharp distribution centered at 1808 ± 196 pN and adhesion frequencies around 33% (Fig. 3, F and G, and fig. S7). The rupture lengths are $\sim 460 \pm 122$ nm, closely matching those observed in the full-protein experiments. These results confirm that p_{PROX} contains the correct binding site within DSG-1 and that a single interaction with SdrD is highly force resilient, remaining stable under nanonewton-scale loads.

Mechanostability and sequential domain unfolding of the SdrD complex

The SMD results showing high resilience to mechanical load for p_{PROX} strongly suggest that it is the binding site for the SdrD A-domain. The SMFS experiments with p_{PROX}^{*} confirmed these finds. However,

looking only at rupture events would be a limited use of the experimental SMFS traces. SdrD⁽⁺⁾:corneocyte experiments exhibited a large number, 91% (2339 of the 2570 curves), of adhesive curves featuring sawtooth patterns with multiple (mostly 2, 3, or 4) equally spaced force peaks preceding the final rupture peak (Fig. 4A). The canonical sawtooth pattern was also detected with SdrD⁽⁺⁾:DSG-1, with unfolding forces and peak-to-peak distances similar to those observed in corneocytes (Fig. 4, B and C). Same can be said about the single-molecule experiments with p_{PROX}^{*} (fig. S8). These profiles reflect the force-induced unfolding of protein secondary structures (α helices and β sheets) (64) and have been reported for the multipeptide staphylococcal adhesins SasG (65) and Aap (66).

The SdrD⁽⁺⁾:corneocyte signatures featured unfolding forces of 2213 ± 267 pN in 2339 adhesion force profiles (nine cell pairs) and a mean peak-to-peak distance of 43 ± 7 nm (Fig. 4B), matching the length expected for fully extended B-domain immunoglobulin-like folds (110 amino acids $\times$ 0.36 nm per residue, 4 nm for the folded domain). A similar behavior was observed for SdrD⁽⁺⁾:DSG-1 (Fig. 4C). Consistent with SMFS experiments on purified SdrG B1 and B2 domains (67), our data collected directly on physiologically relevant bacterial-corneocyte systems demonstrate that the force required to unfold individual SdrD B-domains is extremely high, representing the strongest protein fold reported thus far.

The sawtooth behavior of the force traces presented in Fig. 4A can also be used as a signature of the binding complex. To investigate the role of B-domains in complex stability and reproduce the adhesive sawtooth patterns observed experimentally, we generated tridimensional models for the full-length SdrD:DSG-1 complexes

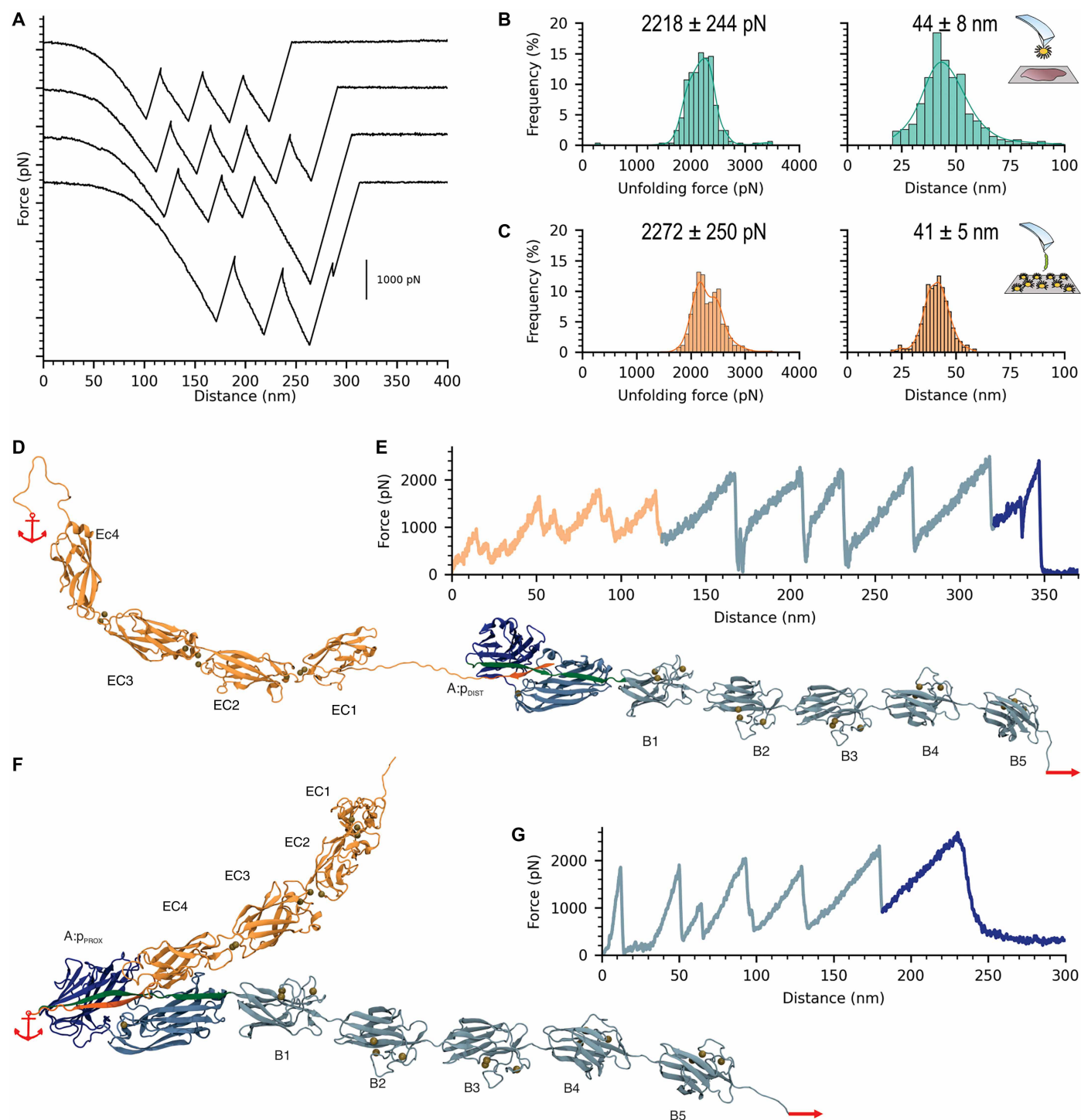


Fig. 4. Adhesin B-domains unfolding forces are equally strong and follow a consistent pattern. (A) Canonical sawtooth pattern detected on SCFS experiments for *L. lactis* SdrD⁽⁺⁾ binding to corneocytes. (B and C) Unfolding forces observed for SCFS of *L. lactis* SdrD⁽⁺⁾ binding to corneocytes and SMFS of *L. lactis* SdrD⁽⁺⁾ binding to DSG-1, respectively. Peak-to-peak distances match the expected length for fully extended B-domains [$n = 337$ and $n = 353$ for (B) and (C), respectively]. (D and F) 3D structure of the full-length model for SdrD:DSG-1 where p_{DIST} or p_{PROX} is at the complex interface, respectively. The different domains are indicated in the image and anchor and pulling points used for SMD simulations are also displayed. Color code for SdrD N2 and N3 domains and DSG-1 is the same as in Fig. 2. B-domains are colored in teal. (E) Representative force-versus-extension curves for the full-length SdrD:DSG-1^{pDIST} model. Curves are colored by protein domain. We observed distinct unfolding patterns for the DSG-1 EC domains, followed by SdrD five B-domains and, lastly, the rupture of the interaction between SdrD N2-N3 and p_{DIST}. (G) Representative force-versus-extension curves for the full-length SdrD:DSG-1^{pPROX} model. Curves are colored by protein domain. We observed distinct unfolding patterns for SdrD five B-domains and, lastly, the rupture of the interaction between SdrD N2-N3 and p_{PROX}, consistent with observed experimentally, corroborating that p_{PROX} is the probable binding site for SdrD.

using a combination of AlphaFold2 (68), to generate the individual DSG-1 EC domains and SdrD B-domains, and MODELLER, to combine it with SdrD A-domain. These models included the five B-domains of SdrD and the four EC domains of DSG-1, considering both binding sites where either p_{DIST} or p_{PROX} is at the SdrD:DSG-1 interface (Fig. 4, D and F). The systems were built with all-atoms and solvated in an explicit water box for geometry optimization and energy equilibration. Due to the complexity and size of the model structures, the production SMD runs were performed at the coarse-grained (CG) level using Martini 3 (69) implemented in GROMACS (70). The pulling simulations were performed by holding on the free C-terminal end of DSG-1 and pulling on the C-terminal end of SdrD. Exemplary traces show multiple peaks in a sawtooth pattern as observed experimentally (Fig. 4, E and G). However, simulations with p_{DIST} as the binding position exhibited extra, lower-force peaks, characteristic of the DSG-1 EC domains unfolding (Fig. 4E). Additionally, the system length exceeded 340 nm, which is too long compared to the experimental traces.

It is important to note that, due to the inherent resolution limits of CG models, particularly their simplified treatment of side-chain interactions and electrostatics, rupture force differences between the two binding modes (p_{PROX} versus p_{DIST}) are not well resolved in the CG force-extension traces. This is an expected limitation, as CG simulations are designed to capture global mechanical responses rather than fine interaction details, which are more accurately represented in aa-SMD simulations and experimental SMFS. As a result, the rupture forces observed in dark blue in Fig. 4 (E and G) appear similar for both complexes, in contrast to the distinct rupture forces reported in Fig. 3D.

For the SdrD:DSG-1 ^{p_{PROX}} model, between three and five main peaks were observed before the main rupture event, depending on the simulation replica. These peaks corresponded to the unfolding of the B-domains and have an unfolding force similar to the rupture force of the A-domain. The force trace profiles obtained for the SdrD:DSG-1 ^{p_{PROX}} complex (Fig. 4G) align with the experimentally observed sawtooth profile, lacking low rupture peaks that could correspond to EC DSG-1 cadherin domains. Instead, we reproduced the five unfolding force peaks preceding the rupture event.

Visual inspection of the MD trajectories provided insights into the sequential rupture of the domains within the complex. The B-domains underwent rupture in a seemingly stochastic order, followed by dissociation at the A-domain interface. The forces required to induce rupture were comparable across all B-domains, with the A-domain exhibiting marginally elevated forces (Fig. 4G). Based on the force profiles, comparison with experimental data, and these findings, we inferred that p_{PROX} is the binding partner for SdrD. Additionally, these results confirm that, as previously proposed (67, 71), the B-domains function as shock absorbers, enhancing the “survivability” of the A-domain under high force loads.

Loading rate-dependent adhesion and mechanostability of the SdrD complex

Next, we investigated the loading rate dependence of SdrD adhesion to both corneocytes and the DSG-1 protein using dynamic force spectroscopy (DFS) with AFM pulling experiments at varying speeds, providing different loading rates (Fig. 5, A and B). Consistent with the BE (72–74) and Dudko-Hummer-Szabo (DHS) models (75), our initial results demonstrated that higher loading rates led to increased rupture forces. Notably, experiments with corneocytes at

high loading rates revealed a population resisting adhesion forces up to 4 nN (Fig. 5A), indicating that mechanical stress enhances bacterial-corneocyte adhesion. Similarly, we observed that the strength of the SdrD–DSG-1 bond also increases with applied mechanical force (Fig. 5B).

The DFS plot of the corneocyte experiments (Fig. 5A) shows that the 2-nN forces fit a BE model with a kinetic off rate (k_{off}) of $8.8 \times 10^{-8} \text{ s}^{-1}$ and a transition energetic barrier at (x_{β}) of 3.40×10^{-2} nm. However, at higher loading rates, we observed a transition to a high-force regime in the 3- to 5-nN range, which exceeds BE model predictions. For the SdrD–DSG-1 interaction (Fig. 5B), the DFS adhesion and unfolding plots fitted the BE model, yielding kinetic parameters with a k_{off} of $1.4 \times 10^{-7} \text{ s}^{-1}$ and a transition energetic barrier at (x_{β}) of 3.96×10^{-2} nm, matching those on corneocytes. At higher loading rates, the high-force adhesion population was even more pronounced with DSG-1.

This shift suggests a mechanical activation of the adhesion complex, allowing it to withstand higher forces under stress. Such force-enhanced adhesion may provide a critical advantage for *S. aureus* during skin colonization, helping it resist mechanical challenges like hydrodynamic flow, cell-surface contacts, and epithelial turnover, confirming that mechanical stress activates a strong SdrD–DSG-1 interaction. While the 3- to 5-nN adhesion forces observed in corneocytes could involve multiple adhesins, this is unlikely for the SdrD–DSG-1 experiments, where DSG-1 was identified as the specific ligand for SdrD. In these conditions, catch-bonds formed by SdrD with DSG-1 strengthen under tension, marking this interaction as the strongest noncovalent bond recorded to date at 10^5 pN/s. This behavior is consistent with previous findings for SdrG using magnetic tweezers (76), but the forces observed here, particularly the 4 nN regime, set a benchmark for noncovalent interactions.

Atomic-level insights into the hyperstability of the SdrD complex under mechanical load

To elucidate how the SdrD:DSG-1 interaction could become more resilient to shear forces than the SdrG:Fg β complex, we used our in silico SMFS approach, which allows us to track every atom of the system during the rupture event. To ensure the computational mechanism matched the experimental observations, we built a DFS using both aa-SMD and CG-SMD simulations (Fig. 5C). A total of 560 replicas were simulated for a combined duration of $\sim 871 \mu\text{s}$ (tables S2 and S3). Simulations were performed on DGX-A100 nodes using NAMD (aa-SMD) and GROMACS (CG-SMD), following established protocols (77). The in silico DFS showed the same trend as the experimental work, with increasing mechanical force resilience for high force regimes (Fig. 5C).

To gain atomic-level insights into the DFS results, we analyzed the aa-SMD trajectories using standard protocols in Visual Molecular Dynamics (VMD) software (78) and the dynamical network analysis approach (79). In MD simulations, dynamical network analysis helps identify communication pathways and correlated motions between atoms or residues in biomolecules. Nodes, typically amino acid residues, are connected by edges representing interactions, which are filtered by distance and weighted based on correlation strength, using mutual information theory to capture communication between nodes (fig. S9). Within this network, force propagation pathways can be visualized (80) between the SMD anchor and the pulling points (fig. S10). Our analysis revealed that most of the force propagates from the protein latch through the peptide, indirectly interacting

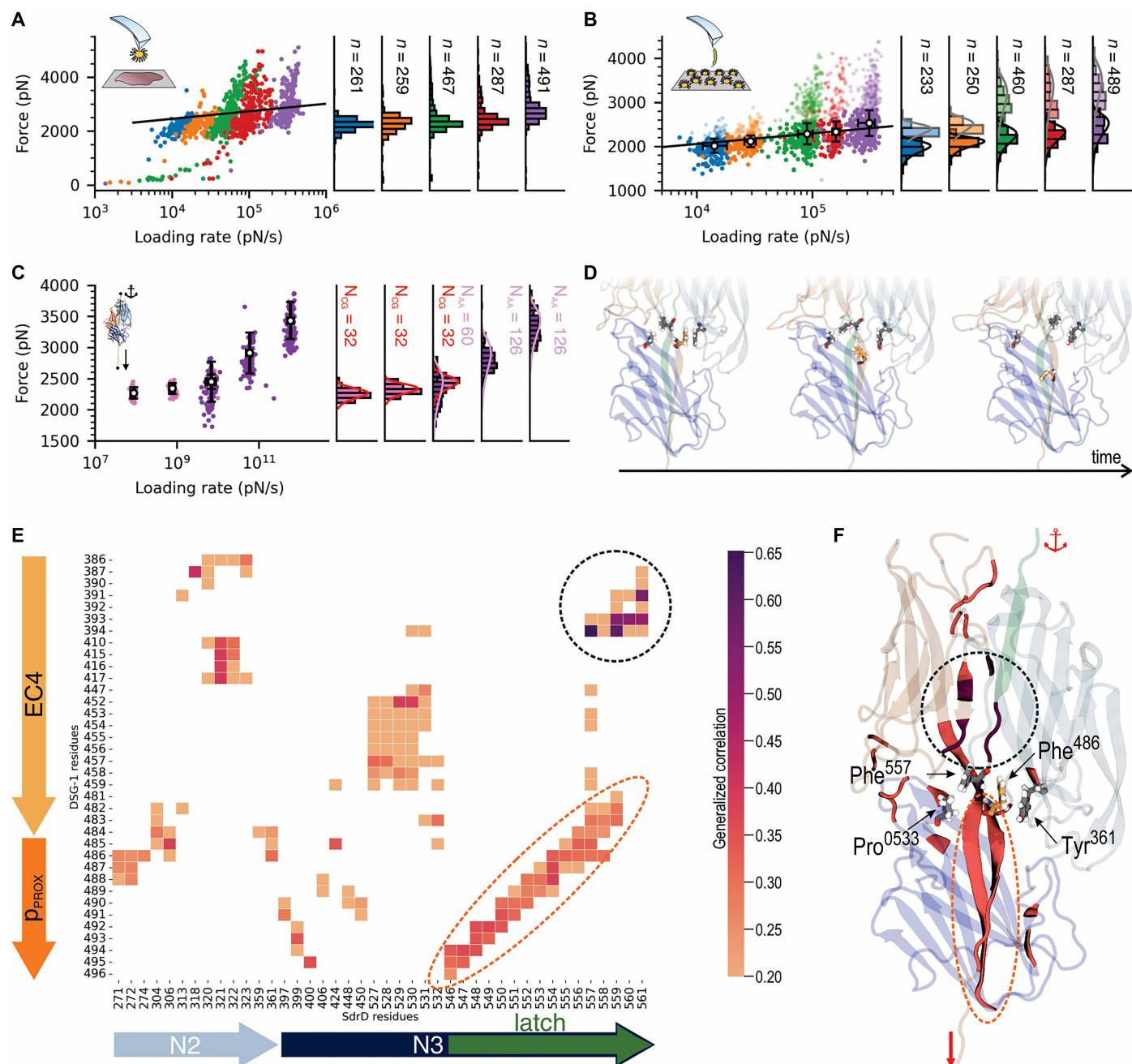


Fig. 5. Bond strength between SdrD and DSG-1 increases as mechanical force is applied. Force-dependent activation is evaluated using DFS in vitro and in silico. (A) Force versus loading rate for SCFS *L. lactis* SdrD⁽⁺⁾ experiments. The 2-nN forces were described by a BE model, with a kinetic off rate (k_{off}) of $8.8 \times 10^{-8} \text{ s}^{-1}$ and a transition energetic barrier at ($x\beta$) of $3.40 \times 10^{-2} \text{ nm}$. (B) Force versus loading rate for SMFS *L. lactis* SdrD⁽⁺⁾ experiments. Adhesion forces were described by a BE model with a k_{off} of $1.4 \times 10^{-7} \text{ s}^{-1}$ and a transition energetic barrier at ($x\beta$) of $3.96 \times 10^{-2} \text{ nm}$, matching those on corneocytes. Only adhesion forces of $>1000 \text{ pN}$ were considered. (C) Force versus loading rate for SMD simulations of SdrD:DSG-1^{pPROX}. As for the experiments, at high loading rates, we observe a sharp shift in force that reflects the activation of the complex by mechanical stress. (D) SMD snapshots showing the aromatic π -stacking interactions at the SdrD:DSG-1^{pPROX} interface. The protein is colored using the same color scheme as in Fig. 3, and residues participating in aromatic interactions are shown as sticks. (E) The generalized correlation-based dynamical network analysis (79) was used to extract correlations of motions between SdrD and DSG-1^{pPROX} interface. The heatmap matrix shows highly correlated interface residues (correlation values > 0.2). (F) SdrD:DSG-1^{pPROX}. Highly correlated residues colored according to the heatmap in (E). We observe that most residues are located on SdrD latch motif and also at EC4 interface with the latch. Residues participating on aromatic interactions are labeled.

with β sheets in the N3 domain. This force transmission pattern differs from those observed in SdrG (35, 36) and Bbp (34), where connections were observed between the latch and the N2 domain.

Community analysis of the network (fig. S11) highlights key amino acid residues with strong interconnections. In dynamical network analysis, communities are groups of tightly connected nodes (residues) that interact more with each other than with the rest of the network. Identifying these communities reveals functional regions within biomolecules, such as domains or allosteric sites. In our case, the latch and N2 domain were grouped in the same community (purple), although the connection was less pronounced than what was previously observed for SdrG and Bbp. We identified a unique community that links the latch and EC4, as well as a central community connecting the peptide, latch, and N3 domain (salmon). This previously unidentified pattern of highly connected residues between the peptide, latch, and cadherin EC4 domain supports the hypothesis that EC4 enhances the rigidity and stability of the complex under high-force loads. These findings are further supported by the network betweenness analysis (fig. S12). Betweenness is a measure of the importance of a node (residue) in facilitating communication within the network. High betweenness values indicate that a residue plays a critical role in connecting different regions of the protein. In our analysis, the strong correlation between the N2 and N3 domains with EC4, as indicated by the betweenness values, underscores the pivotal role of EC4 in maintaining the structural integrity of the complex under mechanical stress.

To investigate further which residues contribute the most for the hyperstability of the complex, we can plot the pairwise residue interactions at the complex interface (Fig. 5, E and F). Previous studies have shown that SdrG:Fg β exhibits catch-bond behavior (35, 76), which is also expected for SdrD:DSG-1. Highly connected interfaces have been associated with binding energy and also with mechanical resilience properties (39, 40). Our results show that the highest correlated residues are located at the interface between SdrD and the EC4 domain, followed by interactions between the SdrD latch and pPROX (Fig. 5E and fig. S13).

Visual inspection of the SMD trajectories revealed that SdrD residues Tyr³⁶¹, Pro⁵³³, and Phe⁵⁵⁷, along with DSG-1 residue Phe⁴⁸⁶, are involved in Π -stacking interactions that persist until just before the complex ruptures (Fig. 5D). Additionally, Pro⁵³³ and Tyr³⁶¹ are structurally aligned with key residues from the ClfB binding site, which are important for ligand binding (33), and both are highly connected at the ligand interface. In all, the simulations revealed that, unlike the previously studied SdrG:Fg β interaction, which is primarily dominated by backbone-backbone hydrogen bonds, the SdrD:DSG-1 complex is further stabilized by Π -stacking of aromatic residues as well as interactions between EC4 and SdrD.

Calcium's role in enhancing the mechanostability and adhesion of SdrD complex

Another important aspect of the adhesion mechanism is its known calcium dependence (81, 82). Protein folds or complexes with nanonewton-range mechanostability typically include calcium in their structure (82–84). For adhesins, B-domains are known to be highly dependent on calcium, with their resilience to mechanical unfolding becoming negligible when calcium ions are depleted (67). Similarly, we observed that calcium plays a crucial role in the mechanostability of the SdrD B-domains. SdrD binds calcium ions through its B1–B5 domains (31, 32). Ca²⁺ binding induces conformational

changes in SdrD, proposed to regulate the distance between the bacterial surface and the A-domain in a spring-like fashion, ensuring optimal ligand-binding. Here, we assessed the extent to which calcium modulates the mechanical properties of SdrD using SMFS with DSG-1-modified tips. Unfolding forces (sawtooth patterns) associated with the B-domains in normal conditions (Fig. 6A) were abolished (unfolding frequency dropping from 91 to 5%) when calcium was removed via chelation (1 mM EDTA) (Fig. 6B). Adding 10 mM Ca²⁺ restored the sawtooth patterns (Fig. 6C), and the high concentration of Ca²⁺ created a more stable pattern, indicating the role of Ca²⁺-binding sites in each B-domain.

The adhesion frequency in these experiments was not negatively affected by Ca²⁺ depletion (Fig. 6D), and the adhesion force of 2156 ± 210 pN and rupture length associated with the A-domain were not altered (Fig. 6E and fig. S14). The addition of 10 mM Ca²⁺ showed an unfolding frequency of B-domains similar to the initial case but markedly different from the system where calcium was depleted (Fig. 6F). We speculate that, under standard conditions, SdrD binds calcium and possibly other ions present in the growth medium in low amounts, whereas adding 10 mM Ca²⁺ following chelation enables all B-domain binding sites to interact with this ion, contributing to their optimal folded conformation, which is illustrated by the full unfolding signature (five peaks + one adhesion peak). Our observation that Ca²⁺-binding sites control the mechanostability of SdrD B-domains is consistent with SMFS on purified B1 domains from the structurally related SdrG protein.

Additionally, we found that Ca²⁺ greatly enhances the binding properties of the A-domain, with the adhesion frequency and force increasing from 49 to 71% and 2076 ± 248 to 2601 ± 163 pN (658 adhesion force profiles; seven different cells), respectively (Fig. 6, D and E, and fig. S15). This unexpected finding suggests that Ca²⁺ not only governs the stability of the SdrD B-domains but also enhances the ligand-binding strength of the A-domain. Previous studies showed that calcium enhances the stability of B-domains but had little effect on increasing A-domain binding strength (67). To investigate the role of B-domains in stabilizing the A-domain, we performed CG-SMD simulations where we sequentially removed B-domains. Results showed no major differences in A-domain rupture forces regardless of the number of B-domains present (Fig. 6G). Visual inspection of the aa-SMD trajectories revealed that the EC4 cadherin creates a bulk resistance point when dissociating SdrD:pPROX under force. Therefore, we hypothesize that EC DSG-1 cadherin domains, stabilized by calcium ions, likely contribute to the increased binding strength in the A-domain. Between two adjacent cadherin domains, calcium ions stabilize the complex in a relatively rigid structure (Fig. 6H) (85). To test this, we created a toy system with pPROX, EC4, and EC3 cadherin domains, pulling pPROX through a fixed nanopore to simulate cadherin unfolding under force and constriction like when pPROX is bound to SdrD A-domain (fig. S16). Our results show that calcium-depleted cadherins are about 35% easier to unfold than those with calcium-bound sites (Fig. 6I), explaining the increased adhesion force observed experimentally.

Elevated calcium concentrations in AD enhance SdrD-mediated adhesion, increasing bacterial binding across corneocyte surfaces

The increased mechanostability provided by Ca²⁺ may have important clinical implications, particularly in AD, where elevated Ca²⁺ concentration gradients are observed in affected cells. Filaggrin

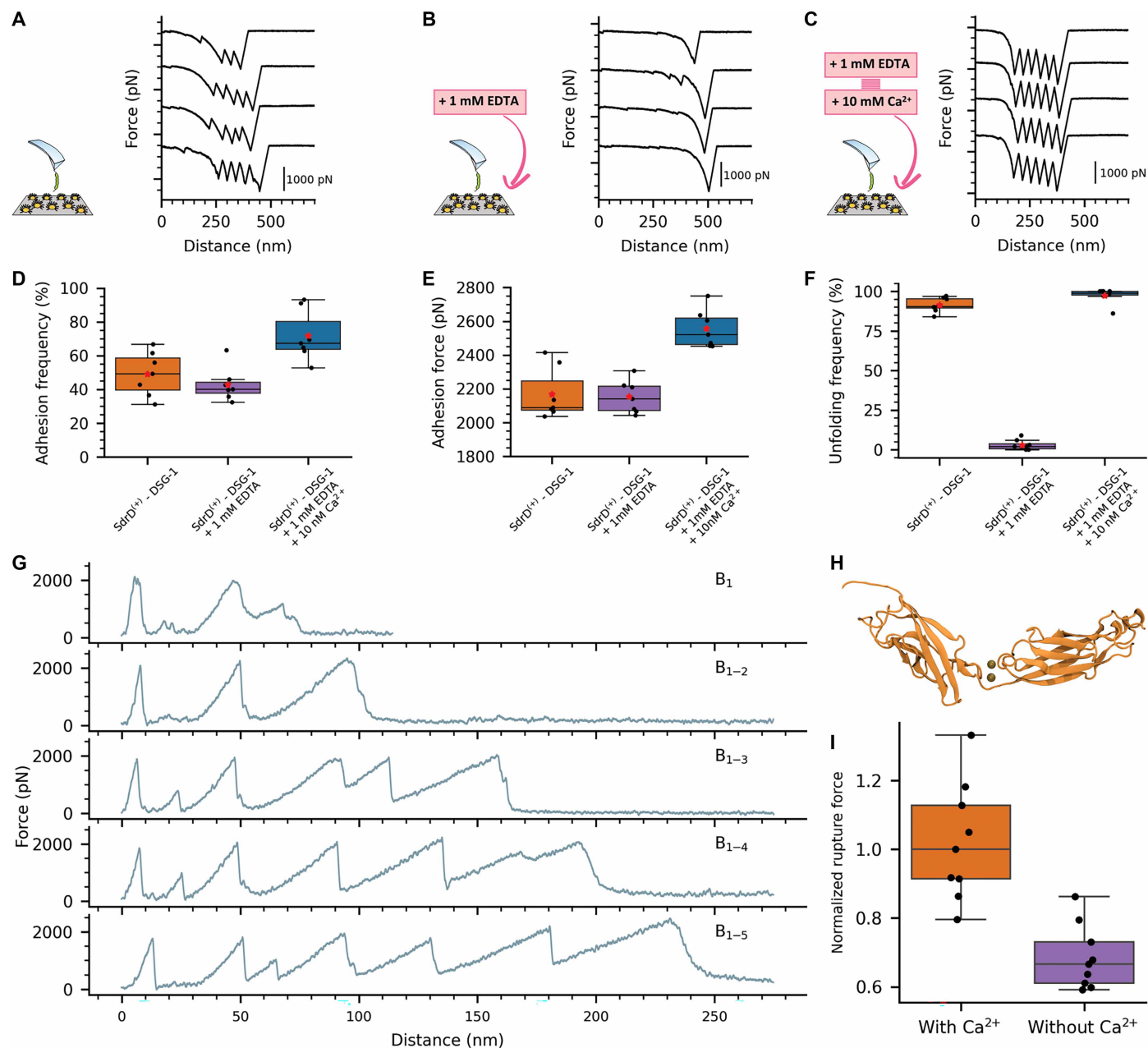


Fig. 6. Calcium plays a crucial role in SdrD A-domain mechanostability. The mechanoproperties of SdrD are modulated by calcium ions. As before, SMFS experiments using AFM tips functionalized with DSG-1 interacting with *L. lactis* SdrD⁽⁺⁾ were performed in presence of calcium (A), when calcium was removed through chelation (B) and also in further addition of a higher calcium concentration (C). (D) Adhesion frequencies (D) and adhesion forces (E) observed for the experiment in a higher calcium concentration were unexpectedly higher. Unfolding forces associated with the B-domains were abolished when calcium was removed (F). Events with no adhesion were not considered on the boxplots; only adhesion forces of >1000 pN were considered. (G) Force-versus-extension curves for SMD simulations where SdrD B-domains were sequentially added to the protein structure. We notice no substantial changes on the rupture forces of the last peak, associated with breaking the interaction between SdrD and DSG-1^{pPROX}, which leads us to believe that the calcium associated with DSG-1 EC domains, instead of the ones present on SdrD B-domains, might be involved on the experimentally observed increase on the adhesion forces. (H) Schematic representation of a toy system created to evaluate the role of DSG-1 EC domain calcium ions. EC3 and EC4 3D structure is represented in cartoon while calcium ions that stabilize the interface are represented as tan spheres. (I) Normalized unfolding forces measured over SMD simulations where we pull EC4 through a constricted pore region to mimic the bulk resistance offered by EC4 adjacent to p_{PROX} with our aa-SMD simulations. We observe a major difference on the unfolding forces in presence of calcium ions at the interface stabilizing the EC3:EC4 interaction, suggesting that both calcium and the location of the binding site might be playing a role in the complex hyperstabilization. Red stars on (D) to (F) represent average points.

deficiency, a common feature in AD, leads to epidermal barrier dysfunction, reduced natural moisturizing factor levels, and abnormal distribution of corneodesmosomes across the corneocyte surface (86). Disruption of the epidermal barrier alters the calcium gradient, likely intensifying Ca^{2+} -dependent interactions. Calcium gradients are also known to play a crucial role in epidermal differentiation (87).

To explore the influence of Ca^{2+} and the molecular effects of SdrD adhesion in AD corneocytes, we conducted a new set of SCFS experiments (Fig. 7A). Previous studies have shown that AD corneocytes exhibit abnormal behavior of corneodesmosomal proteins, such as CDSN, which becomes diffused across the cell surface rather than remaining confined to the periphery (88). Immunofluorescence microscopy has also revealed an abnormal distribution of DSG-1, forming dense, diffuse patterns across the corneocyte surface (18). To test our hypothesis that DSG-1 follows a similar distribution pattern when interacting with *S. aureus* SdrD, we repeated the SCFS experiments using AD corneocytes (Fig. 7A). As in the previous experiments with *L. lactis* SdrD⁽⁺⁾ (Fig. 4A) binding to healthy corneocytes, the force measurements displayed the characteristic sawtooth pattern (Fig. 7B).

The first notable difference between healthy and AD samples was the texture of their surface. Topographic images of AD corneocytes (Fig. 7C) revealed small, circular protrusions, a few hundred nanometers in size, termed circular nano-objects (89), which are hypothesized to correspond to corneodesmosomes (90). The second clear distinction was in the distribution of adhesive events. While *S. aureus* primarily adhered to the edges of healthy corneocytes, it was able to target the entire surface of AD corneocytes (Fig. 7D). This distribution of adhesion events supports our hypothesis, as there is a clear correlation between the location of corneodesmosomes and the adhesion events. Another key observation was the increase in adhesion rupture forces (Fig. 7E and fig. S17). A distinct distribution was noted, with one peak centered at 2156 pN and a second peak around 4000 pN. Rupture lengths were $\sim 321 \pm 50$ nm, consistent with the length of the adhesin (Fig. 7E and fig. S17).

Last, we performed SMFS experiments to investigate the interaction between DSG-1 and *S. aureus* clinical isolates. We used the AD08 strain, which was isolated from the infected skin lesion of a patient with AD (Fig. 8A) (28, 91). The DSG-1:AD08 interaction exhibits two distinct rupture force populations, with a main peak centered at 1546 ± 307 pN, similar to what was observed for *L. lactis* expressing SdrD, and rupture lengths between 284 ± 142 nm, consistent with the expected size of the adhesin (Fig. 8C and fig. S18). Additionally, we observed characteristic sawtooth patterns indicative of SdrD B-domain unfolding, with peak-to-peak distances of ~ 40 nm (Fig. 8B). The unfolding force and extension profiles closely match those seen in DSG-1:SdrD⁽⁺⁾ *L. lactis* interactions (Fig. 8D), strongly supporting that SdrD is the adhesin mediating binding to DSG-1 in the AD08 strain.

DISCUSSION

Our study reveals the mechanism by which *S. aureus* uses the SdrD protein to bind corneocytes, identifying DSG-1 as a key molecular target (Fig. 2). Using a combined experimental and computational biophysics approach, ranging from physiologically relevant pathogen-skin interactions to atomistic molecular analyses, we identified two potential binding sites on DSG-1. We confirmed that SdrD specifically interacts with the region between the transmembrane and EC4

domains of DSG-1 (Fig. 3), as demonstrated by both in silico predictions and experimental SMFS assays. This interaction displays remarkable strength, which is further enhanced under mechanical load. The results are consistent with catch-bond behavior, where the bond lifetime increases in response to applied force.

Notably, the mechanostability of the SdrD complex under high Ca^{2+} concentration exceeds that of previously known SdrG interactions, making it the strongest protein-protein interaction reported to date (Fig. 5). The pivotal role of the EC4 cadherin domain in this enhanced stability provides previously unidentified clues into the structural dynamics of bacterial-human adhesion. Unlike SdrG, which primarily depends on backbone-backbone hydrogen bonds (35), SdrD uses additional interactions with DSG-1's EC4 domain and a π -stacking of amino acids near its latch (Fig. 5). Key residues involved in these interactions, such as Tyr³⁶¹, Pro⁵³³, and Phe⁵⁵⁷ in SdrD and Phe⁴⁸⁶ in DSG-1, are crucial for maintaining the integrity of the complex. This structural arrangement contributes to the exceptional strength of the SdrD:DSG-1 binding.

The critical contribution of the E-cadherin domains to complex stability was confirmed by both in vitro and in silico SMFS experiments. In both approaches, a truncated DSG-1 peptide lacking the EC4 domain was still able to bind to SdrD; however, the rupture forces were significantly reduced compared to the full-length protein (fig. S19). Notably, even in the absence of the E-cadherin domains, the complex resisted forces in the nanonewton regime. These results underscore a key distinction from SdrG, which binds exclusively to a similar peptide region, demonstrating that SdrD enhances its binding strength through a dual mechanism: forming additional contacts with the EC4 domain and establishing a stabilizing π -stacking interaction near its latch. This extended interaction network appears to be essential for the exceptional mechanostability of the SdrD:DSG-1c complex.

Furthermore, calcium ions are essential for the proper folding and stability of many MSCRAMMs, including SdrD (26, 32, 33, 67, 92, 93). In this work, we showed that calcium ions play a fundamental role in SdrD complex stability by stabilizing the E-cadherin domains. Specifically, Ca^{2+} reinforces the EC3:EC4 interface, which, in turn, strengthens the EC4 fold and enhances the overall stability of the SdrD:DSG-1 complex (Fig. 6). Our experiments demonstrated that calcium depletion significantly weakened the mechanostability of the complex, while reintroducing Ca^{2+} not only restored but also further enhanced the bond's strength. Given that calcium gradients play a crucial role in epidermal differentiation, Ca^{2+} may contribute to increased virulence of *S. aureus* infections under certain cell conditions. In damaged skin, the disrupted barrier alters the calcium gradient, potentially amplifying these interactions and bacterial adhesion capabilities (87).

Beyond its role in host protein integrity, calcium is also known to be important for bacterial virulence more broadly (94). Disrupting calcium binding in these proteins could weaken the structure and function of multiple adhesins simultaneously, thereby reducing *S. aureus* adhesion and colonization. In addition to that, monoclonal antibodies targeting DSG-1's EC domains, especially EC4, could serve as competitive inhibitors that block SdrD binding without compromising DSG-1's role in host adhesion. Similar therapeutic approaches have been explored against other bacterial adhesins, such as anti-ClfA antibodies designed to prevent *S. aureus* binding to fibrinogen. While these strategies have shown limited success in clinical settings (95–97), targeting DSG-1 might offer a more specific

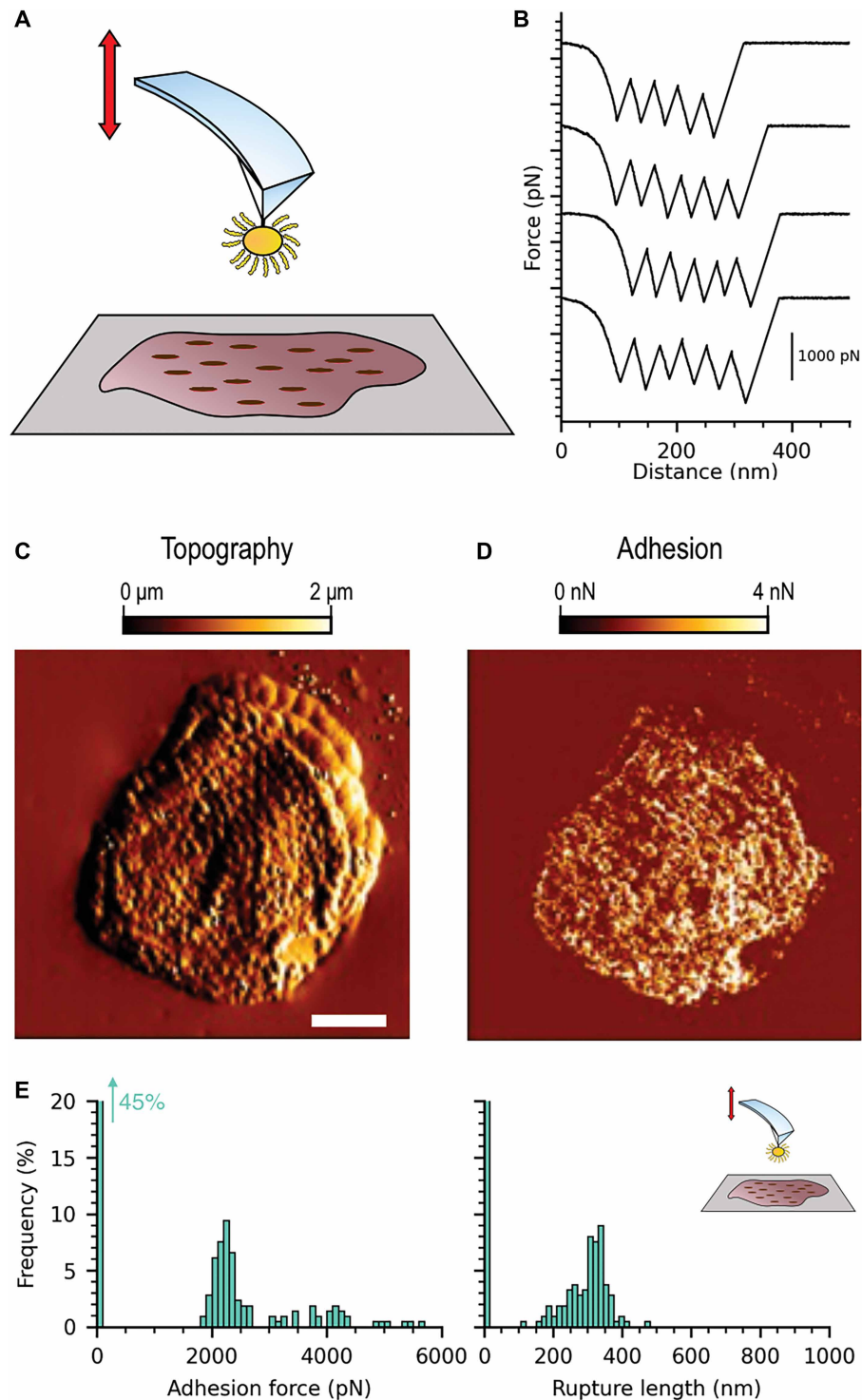


Fig. 7. SdrD mediates bacterial adhesion to AD corneocytes. (A) Schematic illustration of SCFS experiments, such as in Fig. 1A. This time, we mapped the molecular interactions between single *L. lactis* SdrD⁽⁺⁾ bacterial probe and corneocytes from the SC of subjects with AD. (B) Canonical sawtooth pattern detected on SCFS experiments for *L. lactis* SdrD⁽⁺⁾ binding to AD corneocytes. (C and D) Representative topographic images of the structure and adhesion of AD corneocytes, respectively. Different from healthy corneocytes, the strong rupture events (bright pixels) cover the entire cell surface. (E) Adhesion rupture forces were extremely strong, with a sharp distribution centered at 2156 pN and a second one around 4000 pN. Rupture lengths are around 315.8 nm. A representative histogram is shown ($n = 212$).

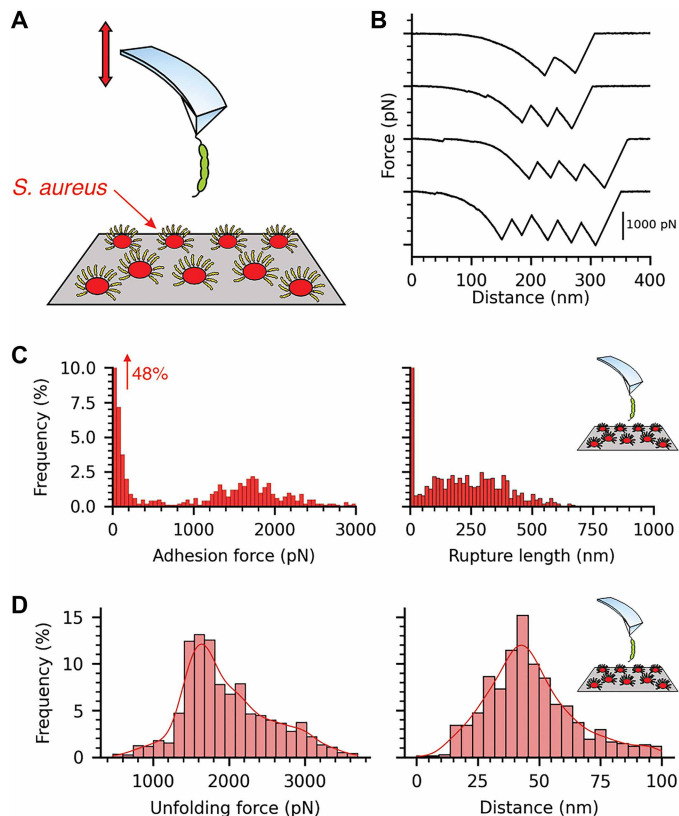


Fig. 8. *S. aureus* AD08 strain isolated from a patient with AD shows ultrastrong forces. (A) Schematic illustration of SMFS experiments where we mapped the molecular interactions between *S. aureus* AD08 and AFM tips functionalized with DSG-1. (B) Similar to *L. lactis* SdrD⁽⁺⁾, sawtooth patterns reflecting unfolding of SdrD B-domains were observed. (C) Adhesion rupture forces were equally extremely strong, with a sharp distribution around 1546 pN, and rupture lengths are around 200 to 300 nm, consistent with the adhesin length. A representative histogram is shown ($n = 1019$). (D) Unfolding force and distance for the *S. aureus* AD08:DSG-1 SMFS experiments. ($n = 1234$).

and accessible intervention point, especially in skin conditions like AD where SdrD adhesion is more prominent. This highlights the potential for targeting calcium-dependent interactions as a broader antimicrobial strategy.

At the tissue level, our findings demonstrated that *S. aureus* exploits the altered skin environment in AD to enhance adhesion and possibly increase colonization by *S. aureus*. SCFS experiments with AD corneocytes revealed increased adhesion frequencies at higher rupture forces, likely due to the abnormal, diffuse distribution of DSG-1 on the cell surface (Fig. 7). The same experiments were performed with *S. aureus* AD08 strain, confirming the role of SdrD:DSG-1 interaction on a clinically relevant bacterial strain (Fig. 8). While *S. aureus* is not the primary cause of this disorder, it is the most common pathogen associated with AD-related infections, exacerbating the condition. This worsening effect is likely driven by increased SdrD binding to the corneocyte surface, facilitated by the disrupted corneodesmosomal architecture.

In summary, our study provides a comprehensive understanding of the molecular mechanisms underlying *S. aureus* adhesion to human skin, identifying DSG-1 as a critical ligand for SdrD. We demonstrated the remarkable catch-bond behavior of this interaction

under mechanical stress and the essential role of calcium in stabilizing the complex. Additionally, the enhanced adhesion to AD corneocytes reveals how *S. aureus* exploits compromised skin conditions to strengthen its colonization at the molecular level. These findings not only expand our knowledge of bacterial adhesion but also open possible avenues for targeted therapies against *S. aureus* infections and skin disorders like eczema. Our study highlights the discovery of the strongest known protein-protein interaction, driven by a previously unknown, calcium-enhanced DLL mechanism, representing a major advancement in the field of bacterial pathogenesis and mechanobiology.

METHODS

Experimental setup

Bacterial strains and growth conditions

Stationary phase cultures of *L. lactis* strain MG1363 carrying the pKS80 plasmid containing the SdrD gene, pKS80::sdrD [SdrD⁽⁺⁾] (98), or *L. lactis* strain transformed with the empty pKS80 plasmid [SdrD⁽⁻⁾] (98) were obtained from Foster and coworkers (98). Cultures were grown overnight in brain heart infusion (BHI) media in the presence of erythromycin (10 μ g/ml) with shaking (180 rpm) at 30°C. Bacteria were then collected by centrifugation (5 min at 2000g), washed twice with phosphate-buffered saline (PBS), and resuspended in PBS, with a 50-fold dilution. A small amount (50 μ l) of the suspension was then deposited on the left side of a polystyrene petri dish, and the cells were allowed to adhere for 10 min then washed with PBS. Confirmation that functional SdrD was present on the surface of *L. lactis* pKS80::sdrD was achieved using in vitro adherence assays with DSG-1, (see fig. S2). *L. lactis* pKS80 served as negative control. The experiment was performed in three biological replicates; a multicomparison using two-way analysis of variance (ANOVA) statistical test was performed with a Šidák posttest ($*P < 0.05$).

Corneocytes collection

Healthy and AD corneocytes were collected using a tape stripping method. Tape strips were collected from healthy participants aged 18 to 65 or pediatric patients with AD aged under 18 years. Sex, gender, age, or ethnicity was not recorded. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Research Ethics Committee of Children's Health Ireland at Crumlin, Dublin, Ireland (ref. 2020402). The collection of tape strips from children with AD was approved by the Research Ethics Committee of Our Lady's Children's Hospital, Crumlin, Dublin, Ireland. Written informed consent from parents/guardians and patient assent (where feasible) was obtained before study enrolment. A clinically unaffected site on the volar forearm was used for SC sampling. Circular adhesive tape strips (3.8 cm²; D-Squame; Monaderm, Monaco, France) were attached to the skin and pressed for 10 s using a D-Squame pressure device (D500; CuDerm, Dallas, TX) with a constant pressure (225 g/cm²) and then removed using tweezers. Eight consecutive tape strips were removed from the same site and immediately stored at -70°C until analysis. The sixth and seventh tape strips were used for this study. On the right side of the dish, a piece of tape strip (5 mm by 5 mm) was immobilized using double-sided tape. The petri dish was filled with 3 ml of PBS.

AD08 collection

S. aureus AD08 is a clonal complex isolate 1 isolated from an infected skin lesion of a patient with AD (99). Overnight cultures of *S. aureus* were grown in 10 ml of tryptic soy broth (TSB) with

shaking (180 rpm) at 37°C. Bacterial cells were harvested via centrifugation (2000g for 5 min) and washed once with TSB medium. They were adjusted to an optical density at 600 nm (OD_{600}) of 0.05 and cultured until reaching the late exponential phase ($OD_{600} = 0.8$) in TSB. Last, the cells were harvested by centrifugation and prepared as described for *L. lactis*.

Single-cell force spectroscopy

Colloidal probes were prepared as described below. Under the control of a NanoWizard III atomic force microscope (JPK Instruments) coupled to an optical microscope, a triangular-shaped tipless cantilever was brought manually into contact with a small droplet of ultraviolet (UV)-curable glue, spread on one side of a glass slide, and then approached to catch a single silica microsphere (6- μ m diameter, Bangs Laboratories Inc.) deposited on the other side of the glass side. The colloidal probe was then exposed to a UV lamp for 15 min to cure the glue. Last, to be able to electrostatically immobilize a single bacterial cell, the colloidal probe was incubated in a 10 mM tris-HCl buffer solution (pH 8.5) containing dopamine hydrochloride (4 mg ml⁻¹) for an hour and washed in the same buffer. The nominal spring constant of these cantilevers was determined by the thermal noise method, giving an average value of ~ 0.08 N m⁻¹. To perform the AFM experiment, the colloidal probe was first brought into contact with a single isolated bacterium (localized by optical microscopy) to catch it electrostatically through dopamine. The obtained so-called cell probe was then positioned over the corneocyte surface. Force-distance curves were collected in QI mode using a constant speed of 30 μ m s⁻¹, a ramp length of 1 μ m, an applied force of 500 pN, a dwell time of 0 s and a Z closed loop. Maps of 256 $\times$ 256 pixels were recorded on 50 μ m-by-50 μ m areas of the corneocyte surface at room temperature. Then, force-distance curves were collected in FV mode using a constant approach and retraction speed of 1 μ m s⁻¹ and 0.5 to 10 μ m s⁻¹ (DFS), respectively, a ramp length of 1 μ m, an applied force of 250 pN, a dwell time of 0 s, and a Z closed loop. Maps of 16 $\times$ 16 pixels were recorded on 5 μ m-by-5 μ m areas of the corneocyte surface (usually, on two different spots of the surface, to account for variability in ligand coverage) at room temperature. Number of cells and details on the analyzed data are included at table S1.

Functionalization of tips with DSG-1

Gold cantilevers (PNP-TR probes, Pyrex Nitride Probe with TRIangular Cantilevers, from NanoWorld) were immersed overnight in an ethanol solution containing 1 mM of 10% 16-mercaptododecahexanoic acid/90% 1-mercapto-1-undecanol (Sigma-Aldrich), rinsed with ethanol, and dried with N₂. Cantilevers were then immersed for 30 min into a solution containing *N*-hydroxysuccinimide (NHS; 10 mg ml⁻¹) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (25 mg ml⁻¹) (Sigma-Aldrich) and rinsed with ultrapure water. Last, they were incubated with recombinant human DSG-1 (0.1 mg ml⁻¹; Gentauro) for 1 hour, rinsed further with PBS buffer, and then immediately used without dewetting.

Functionalization of tips with p^{PROX}

The MSCT-D cantilevers (Bruker) were first amino-functionalized using the gas-phase method of reaction with 3-aminopropyltriethoxysilane as reported previously (100). The functionalization was done as previously described (48, 101, 102). Briefly, the amino-functionalized cantilevers were initially immersed for 2 hours in a reaction solution prepared by dissolving 1 mg of NHS-PEG27-maleimide (JKU, Linz, Austria) in 0.5 ml of chloroform and mixed with 30 μ l of triethylamine. Following immersion, the cantilevers were rinsed with chloroform

and dried under an argon stream. The cysteine-bearing peptide INI-NIQSFGNDDRTNTEPNGSGSGSGSC, here called p^{PROX}, is based on p^{PROX} sequence, considering the amino acids in direct contact with SdrD's latch domain. The peptide was procured from GenScript. A 100- μ l solution of p^{PROX} (100 μ g ml⁻¹) was sequentially mixed with 2 μ l of EDTA (100 mM, pH 7.5), 5 μ l of Hepes (1 M, pH 7.5), 2 μ l of tris(2-carboxyethyl)phosphine (TCEP) hydrochloride (100 mM), and, lastly, 2 μ l of Hepes (1 M, pH 9.6). This prepared mixture was then dispensed onto the cantilevers. After a subsequent 2-hour incubation, the cantilevers were thoroughly washed three times with PBS.

Single-molecule force spectroscopy

A 100 $\times$ diluted overnight bacterial suspension (50 μ l) was deposited on a polystyrene petri dish, and the cells were allowed to adhere for ~ 15 min before being washed with PBS and the petri dish being filled with PBS, similar to SCFS experiments. DSG-1-functionalized gold cantilevers, initially calibrated by the Thermal tune method ($k \sim 0.06$ N m⁻¹), were then brought into contact with the surface of isolated single bacterial cell to record force-distance curves in the AFM force volume mode. Measurements were performed at room temperature with a NanoWizard III or IV AFM. We recorded 32 pixel by 32 pixel maps on areas of 0.5 μ m-by-0.5 μ m on the bacterial surface, with a constant approach and retraction speed of 1 μ m s⁻¹, a ramp length of 1 μ m, an applied force of 250 pN, a dwell time of 0 s, and a Z closed loop, exactly as in SCFS measurements. For loading rate experiments, the retraction speed was varied: 0.5, 1, 3, 5, and 10 μ m s⁻¹. The number of cells and details on the analyzed data are included at table S1.

Calcium experiment

After the recording of maps in the normal conditions, SMFS experiments were repeated in presence of 1 mM EDTA (Sigma-Aldrich) in the medium after 15 min of incubation. Then, a CaCl₂ solution (Sigma-Aldrich) was injected at a final concentration of 10 mM in the medium. After 15 min of incubation, similar force-distance curves were recorded. Number of cells and details on the analyzed data are included at table S1.

Data analysis

Initial data analysis was carried out with data processing software from JPK Instruments (Berlin, Germany). Unfolding and adhesion peaks were well fitted using the worm-like chain model. All plots and final analysis were performed with in house python scripts. Adhesion events were considered for all measurements with force values of >0 pN, unless mentioned. Kernel density estimators were applied to fitting of unfolding forces and distances (Figs. 4, B and C, and 8D). The BE model (72, 74) was used to fit SCFS and SMFS experiments (Fig. 5, A and B); all the fitted parameters are available in the text or figure legends.

Molecular modeling of the SdrD:DSG-1 interaction

Protein sequences

The protein sequences for the SdrD and DSG-1 protein EC1 to EC4 were obtained from UniProt (103) IDs Q2G0L4 (isoform 1) and Q02413, respectively.

Protein structure prediction

The prediction of the apo tridimensional structure of the DSG-1 EC domains (residues 1 to 500) was performed using AlphaFold2 (104). Twenty-four models were generated and optimized using AMBER (105) under the AlphaFold2 protocol. Other parameters were assigned as default. The models were ranked by the per-residue confidence

metric called the predicted local distance difference test score (106), and only the top ranked model was used for further analysis. The full apo tridimensional structure of SdrD (residues 1 to 1349) was also predicted by AlphaFold2 using the same protocol described above

Prediction of the SdrD binding site on DSG-1 structure

The 3D structure model of DSG-1 revealed two loop regions, e.g., with no defined secondary structure: (i) region 1: at the N-terminal loop (residues 1 to 42); and (ii) region 2: between the membrane and the EC4 (residues 485 to 548). The secondary structure propensity to form β strand was calculated for both regions using JPred4 server (107). From region 1, we selected the residues 25 to 42, here called distal peptide or p_{DIST} . The secondary structure prediction for region 2 did not reveal any propensity to form strands or any defined secondary structure. Because this could happen once the peptide is already bound to the adhesin, we used an alternative strategy to select a peptide from the proximal region, here called p_{PROX} . The crystal structure of SdrD in its apo form (33) revealed a similar ligand binding region with the available structures for ClfB. Region 2 protein sequence was then aligned with ClfB available ligands on the PDB (108): dermokine (PDB ID: 4f20), keratin (PDB ID: 4f1z), and Fg α (PDB ID: 4f27). Additionally, we included Fg β complexed with SdrG (PDB ID: 1r17). The sequence alignment was done using MUSCLE (109) with default parameters. Although there is no remarkable sequence similarity between the peptides sequences and DSG-1, they were clustered on the region between residues 485 and 494. One of the Phe residues from Fg β was aligned with Phe⁴⁸⁶ from DSG-1. We then selected p_{PROX} from residues 485 to 500.

SdrD:DSG-1 structure modeling

Attempts to use AlphaFold2 to model the complex between SdrD A-domain (residues 244 to 570) and DSG-1 with either p_{DIST} or p_{PROX} did not yield accurate conformations for the interface region between the latch and the peptide, so we decided for a comparative modeling approach using MODELLER (57). Because the available crystal structures for SdrD are in the apo form (33), an additional template was needed. Protein BLAST (110) was used to search for SdrD homologs against the PDB. SdrG (PDB ID: 1r17) complexed with Fg β was selected as the additional template with good sequence coverage to SdrD (98%) and E -value = 1^{-24} . MODELLER v10.4 was used to build the complex using SdrG and SdrD templates (PDB ID: 4JE0). The calcium ion available at the SdrG crystal structure was transferred to SdrD model. Because Fg β is not an ideal template for DSG-1 peptide, we used MODELLER special restraints function to enforce modeling the DSG-1 peptides as a β strand. Other parameters were assigned as default. A total of 20 models were generated for each system and ranked using the Discrete Optimized Protein Energy (DOPE) score (111). The SdrD: p_{PROX} model also included the EC4 domain, taking in consideration residues 378 to 500.

SdrD:DSG-1 full structure modeling

The 3D structure models for the full sequences of the SdrD:DSG-1 complexes where either p_{DIST} or p_{PROX} at the interface with SdrD A-domain was constructed using a combination of the previously described structures were generated using AlphaFold2 and MODELLER. In each case, the models were superimposed using the measure fit module available with VMD (78). For simplicity, we excluded some disordered regions from SdrD (residues 1 to 243 and 1130 to 1349). Two models were then created: (i) SdrD²⁴⁴⁻¹¹²⁹:DSG-1¹²³⁻⁵⁰⁰: full structure where p_{DIST} is at the complex interface; and (ii) SdrD²⁴⁴⁻¹¹²⁹:DSG-1³⁷⁸⁻⁵⁰⁰: full structure where p_{PROX} is at the complex interface.

To reduce the computational complexity of the system, we included only EC4 domain from DSG-1. Calcium atoms were modeled into the DSG-1 structure using MODELLER, according to the available structure of DSG-3 EC domains (PDB ID: 5eqx) (112). Because the calcium binding residues are highly conserved on adhesin B-domains (33), they were manually superimposed to all SdrD B-domains according to B1 calcium ions available at PDB ID 4JEZ.

aa-SMD simulations

Models of SdrDA-domain:DSG-1 ^{p_{DIST}} and SdrDA-domain:DSG-1 ^{p_{PROX}} were submitted to aa-SMD simulations using NAMD 3 (58) with the CHARMM36 (113) force field. In each case, the complex was solvated using the TIP3 water model (114), and the net charge of the protein was neutralized using a 150 mM salt concentration of sodium chloride. To preserve the models integrity, harmonic restraints were added to SdrD secondary structure through the extraBonds NAMD feature. All MD simulations were performed in periodic boundary conditions, and a distance cutoff of 11.0 Å was applied to short-range nonbonded interactions, whereas long-range electrostatic interactions were treated using the particle-mesh Ewald method (115). Before the aa-SMD, the system was submitted to an energy minimization protocol for 1000 steps. An MD simulation with position restraints in the protein backbone atoms was performed for 1 ns, with temperature ramping from 0 to 300 K in the first 0.5 ns at a time step of 2.0 fs in the NVT ensemble, which served to pre-equilibrate the system. aa-SMD simulations were performed in the NPT ensemble with temperature maintained at 300 K using Langevin dynamics for temperature and pressure coupling, the latter kept at 1 bar. The time step of integration was chosen to be 2 fs for all production runs. aa-SMD simulations were carried out in several replicas, using a constant velocity stretching protocol at different pulling velocities (table S2). SMD was used by harmonically restraining the position of the amino acid at the C terminus of SdrD and moving a second restraint point at the C terminus of DSG-1 peptide, with constant velocity in the z axis with a spring constant of 5 kcal/mol per square angstrom (3.475 N m^{-1}). The force applied to the harmonic spring is then monitored during the time of the SMD. The pulling point was moved with constant velocity along the z axis, and, due to the single anchoring point and the single pulling point, the system is quickly aligned along the z axis. aa-SMD simulations were also performed for the SdrD: p_{PROX}^* complex, using the same parameters as described above.

All-atom equilibrium MD simulations

Before binding energy calculations, the SdrDA-domain:DSG-1 ^{p_{DIST}} and SdrDA-domain:DSG-1 ^{p_{PROX}} models were submitted to equilibrium MD simulations using NAMD 3 with the CHARMM36 force field. System preparation, solvation, minimization, and equilibration runs followed the same protocol described above. Production runs were carried out for 100 ns in eight replicas for each system.

Free energy of binding prediction

The free energy of binding between SdrD and p_{DIST} or p_{PROX} was estimated using the MM-GBSA approach (61). MM-GBSA is based on MD trajectories of the complex. In summary, the free energy of the complex is estimated from the following equation

$$G = E_{\text{bnd}} + E_{\text{el}} + E_{\text{vdW}} + G_{\text{pol}} + G_{\text{np}}$$

where the first three terms are standard MM energy terms from bonded (bond, angle, and dihedral), electrostatic, and van der Waals interactions. G_{pol} is obtained by using the GB model, whereas the nonpolar term is estimated from a linear relation to the solvent

accessible surface area. In MM-GBSA, the entropy contribution to the binding energy is neglected. Implicit GBSA solvent models are used to estimate the solvation energies. MD snapshots were obtained from the last quarter of the MD trajectories. A bootstrap analysis was performed to assess the significance of the difference between the free energy of binding between SdrD and p_{DIST} or p_{PROX} , accounting eight simulation replicas each. The data were resampled 1000 times with replacement to generate bootstrap samples. The bias-corrected and accelerated method was used to construct 95% confidence intervals for the mean difference between the two configurations. The difference between the two samples was estimated to be 3.80 U with 95% confidence interval (CI) of 3.51 for p_{DIST} and 95% CI of 3.51 for p_{PROX} . The SE of the mean difference was calculated to be 0.15 U. The confidence intervals indicate that the mean difference between both peptides configurations is statistically significant.

CG-MD simulations

CG-MD simulations were performed for full models of the complex SdrD:DSG-1 with either p_{DIST} , SdrD²⁴⁴⁻¹¹²⁹:DSG-1²³⁻⁵⁰⁰, or p_{PROX} , SdrD²⁴⁴⁻¹¹²⁹:DSG-1³⁷⁸⁻⁵⁰⁰, at the interface. We have also performed CG-MD simulations for the complexes containing only SdrD's A-domain, SdrD²⁴⁴⁻⁵⁷⁰, and DSG-1 ^{p_{DIST}} or DSG-1 ^{p_{PROX}} + EC4(485-500), for a couple of very slow pulling velocities that would be too computationally expensive to simulate all-atom (table S3). In all cases, the protein complexes were converted onto the Martini 3.0 CG force field (v.3.0.b.3.2) (116) using Martinize2 v0.7.3 (117). A set of native contacts, based on the rCSU+OV contact map protocol, was computed from the equilibrated all-atom structure using the rCSU server (118) and used to determine Gō-MARTINI interactions (119) used to restrain the secondary and tertiary structures with the effective depth (epsilon) of Lennard-Jones potential set to 9.414 kJ mol⁻¹. Gō-MARTINI interaction potential was multiplied by 10 for the protein residues at the interface with p_{DIST} or p_{PROX} . All CG-MD simulations were performed using GROMACS (70) version 2021.5. The full model of SdrD:DSG-1 with p_{DIST} on the interface was placed into a rectangular box measuring 20 nm by 20 nm by 500 nm to the *x*, *y*, and *z* directions. The anchor point was set at the C terminus of EC4 (Gly⁴⁶⁵) and the pulling point was set at the C terminus of B5 domain (Asp⁸⁸⁶). Both points were used to align the protein to the *z* axis. Due to the size of the systems, smaller boxes and different pulling points were sampled to improve the computational time. The full model of SdrD:DSG-1 with p_{PROX} was placed into a rectangular box measuring 15 nm by 15 nm by 350 nm to the *x*, *y*, and *z* directions. The anchor point was set at the C terminus of B5 domain (Asp⁸⁸⁶), and the pulling point was set at p_{PROX} N-terminal residue (Ile¹²³). Both points were used to align the protein to the *z* axis. The box was then solvated with Martini 3 water molecules. Systems were minimized for 10,000 steps with steepest descent, followed by a 10-ns equilibration at the NPT ensemble using the Berendsen thermostat at 298 K, while pressure was kept at 1 bar with compressibility set to 3×10^{-4} per bar, using the Berendsen barostat. A time step of 10 fs was used to integrate the equations of motion. Pulling simulations were subsequently done at the NVT ensemble with a time step of 20 fs. The temperature was controlled using the v-rescale thermostat (120) with a coupling time of 1 ps. For all CG-MD simulations, the cutoff distance for Coulombic and Lennard-Jones interactions was set to 1.1 nm (121), with the long-range Coulombic interactions treated by a reaction field (122) with $\epsilon_r = 15$. The Verlet neighbor search (123) was used in combination with the neighbor list, updated every 20 steps. The LINCS (124) algorithm was used to constrain the

bonds and the leapfrog integration algorithm for the solution of the equations of motion. Several replicas of CG-SMD simulations were performed at a range of speeds described at table S3.

The CG-SMD simulations were systematically rescaled on the basis of the fraction of most probable loading rate and most probable force of the lowest overlapping pulling rate (5.0×10^{-6} nm/ps) between all-atom and CG simulations. Therefore, the CG loading rates and peak forces were multiplied by 6.11 and 1.89, respectively.

Analysis of SMD

For all simulations, the force-extension curves were analyzed by custom python scripts. For dynamic force spectra, the rupture or unfolding forces versus loading rate was plotted, and median forces and loading rates for each pulling speed were fitted to BE model (72, 74) to estimate the effective distance to the transition state (Δx) and the intrinsic dissociation rate or unfolding rate (k_{off}) in the absence of force. The median forces and loading rates at the various pull rates were then used to fit a DHS model (125).

Dynamical network analysis

The generalized correlation-based dynamical network analysis (79) was used to extract correlations of motion between SdrD and DSG-1 interface residues over the aa-SMD simulations. We analyzed all replicas for the slowest pulling velocity (5.0×10^{-6} nm/ps). A network is defined as a set of nodes that represent amino acid residues, and the node's position is mapped to that of the residue's α carbon. Edges connect pairs of nodes if their corresponding residues are in contact, and two nonconsecutive residues are said to be in contact if they are within 4.5 Å of each other for at least 75% of analyzed frames. The mean correlation analysis was carried out on 500 frames before the chosen rupture events at each replica, using a cutoff of 0.2 for the mean correlation coefficients. Binding affinities were calculated using established protocols on the basis of the sum of correlations (126).

Images and plots

All protein structure renderings were performed using VMD (78, 127), along with its plugins and TCL scripts, unless otherwise specified. Graphs were generated using Python 3 libraries, including Matplotlib (128), Pandas (129), and Seaborn (130).

Supplementary Materials

The PDF file includes:

Figs. S1 to S19
Tables S1 to S3
Legend for data S1

Other Supplementary Material for this manuscript includes the following:

Data S1

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