BIOPHYSICS

Force matters in hospital-acquired infections

Extremely strong forces help staphylococci to colonize biomaterials and infect humans

By **Philippe Herman-Bausier**[1] *and* **Yves F. Dufrêne**[1,2]

Bacterial pathogens show a remarkable capacity to stick to host tissues and implanted biomaterials, grow, and form biofilms on these surfaces (*1*). These multicellular communities protect the bacteria from the host immune system and from drugs, thereby causing infections that are difficult to eradicate. Today, biofilms are estimated to be involved in half of all infections acquired in hospitals. On page 1527 of this issue, Milles *et al.* (*2*) combine single-molecule experiments and molecular dynamics simulations to study the forces involved in the adhesion of bacterial pathogens to host proteins, the first step of biofilm formation.

Biofilm infections commonly involve *Staphylococcus epidermidis* and *S. aureus* strains, including methicillin-resistant *S. aureus* (MRSA). These microbes are decorated with adhesins that mediate both attachment to host proteins and cell-cell association (*3*). The structural features and molecular biology of staphylococcal adhesins have been widely investigated, but their binding forces are poorly understood because of a lack of ultrasensitive biophysical force probes. However, recent progress in single-molecule techniques (*4*) has provided new opportunities for studying forces in bacterial proteins (*5*).

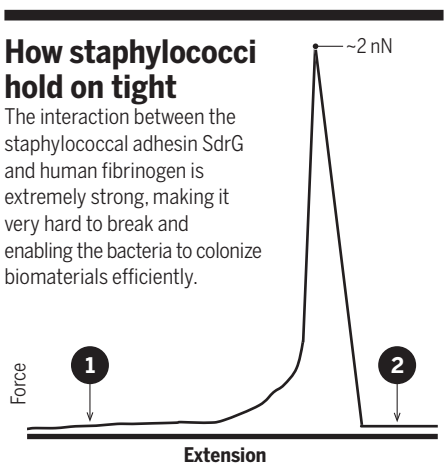
Unlike traditional methods that probe large ensembles of cells and molecules, atomic force microscopy (AFM) makes it possible to study bacterial components one molecule at a time (*5*). The technology has enabled researchers to understand the nanoscale biophysical properties of bacteria, unravel the binding mechanisms of their individual surface molecules, and decipher the forces guiding cell-cell and cell-substrate interactions (*5*).

Milles *et al.* elegantly combine single-molecule AFM and steered molecular dynamics (SMD) simulations to investigate the molecular mechanism by which the prototypical staphylococcal adhesin SD-repeat protein G (SdrG) binds to fibrinogen (Fg), a host protein that rapidly coats implanted biomedical devices. They show that the extreme mechanical stability of the SdrG-Fg complex originates from an intricate hydrogen bond network between the ligand peptide backbone and the adhesin. The study represents an important step toward understanding how hospital-acquired pathogens use their surface adhesins to guide cell adhesion and trigger infections.

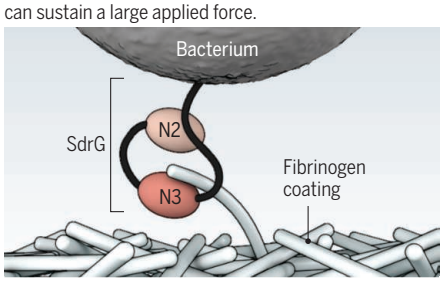
SdrG binds to Fg via a dock, lock, and latch (DLL) mechanism that involves dynamic conformational changes of the protein, resulting in a greatly stabilized adhesin-ligand complex (*6*). The N2 and N3 subdomains of SdrG bind to a short peptide sequence in the Fg molecule (see the figure). Milles *et al.* used single-molecule AFM to quantify the mechanical strength of the SdrG-Fg complex. By immobilizing the SdrG subdomains on the AFM tip and the ligand peptides on a substrate, the authors could probe the forces between the interacting molecules in their native configuration. Consistent with earlier AFM experiments on living bacteria (*7*), these in vitro measurements revealed that the SdrG-Fg interaction is ultrastrong, with a strength of ~2 nN, similar to that of covalent bonds (*8*).

Sophisticated simulations enabled Milles *et al.* to unravel the mechanism behind this extreme mechanostability. They found that the target peptide is confined in a screwlike manner in the binding pocket of SdrG and that the binding strength of the complex results from numerous hydrogen bonds between the peptide backbone and SdrG, independent of peptide side chains. Rupture of the complex requires all hydrogen bonds to be broken simultaneously. The authors observed similar side-chain–independent mechanical stability in experiments and simulations of clumping factor B (ClfB), a protein from *S. aureus* that is structurally and functionally related to SdrG. This finding suggests that this hydrogen bond mechanism may be generalized to other adhesins.

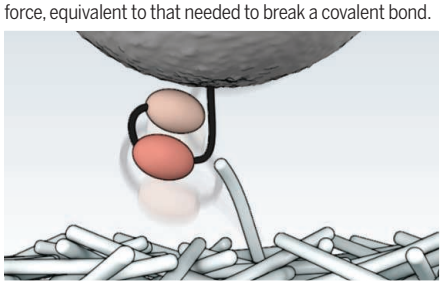
The extreme mechanical stability of SdrG explains at the molecular scale how staphylococci colonize biomaterials so efficiently while sustaining high mechanical stresses (*9*). The 2-nN force is much larger than that reported for other cell adhesion molecules, as well as for the mechanically strong biotin-streptavidin and cohesin-dockerin complexes. However, this ultrahigh binding strength is in contrast with biochemical data showing that SdrG binds its ligand with moderate affinity (*6*). This discrepancy shows that binding forces measured at nonequilibrium are uncorrelated with the equilibrium binding affinity. Given that in nature, most surface-attached bacteria are subjected to physical stresses (*9*), force measurements are likely to be more relevant than traditional bioassays for properly describing cell adhesion. This is particularly true for staphylococci, which are constantly exposed to fluid shear forces during colonization of implanted biomaterials.



1 The N2 and N3 subdomains of SdrG bind to fibrinogen through an intricate hydrogen-bonding network that can sustain a large applied force.



2 The tight binding is only broken by application of a large force, equivalent to that needed to break a covalent bond.



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The authors also speculate that SdrG may bind its ligand through a catch bond—that is, a bond strengthened by tensile force, as observed with the *Escherichia coli* adhesin FimH (*10*). Supporting this idea, a recent single-molecule study demonstrated that the mechanical strength of ClfB increases dramatically as mechanical force is applied (*11*). The results suggested that ClfB-mediated adhesion is enhanced through force-induced conformational changes in the adhesin, which changes from a weakly binding folded state to a strongly binding extended state. This force-dependent ligand-binding mechanism may help *S. aureus* to attach firmly to biomaterials under high shear stress, and to detach under low shear stress to colonize new sites.

The study by Milles *et al.* has important implications for many fields. In molecular microbiology, the combined use of AFM experiments and SMD simulations should greatly contribute to the identification of new binding mechanisms in bacterial adhesins, thus helping to show how they regulate biofilm formation. In diagnosis and therapy, this combined approach could represent a powerful platform for the treatment of microbial infections. For instance, correlative single-molecule experiments and simulations could be used to screen antiadhesion compounds for their potential to prevent or treat biofilm-associated infections (*12*). The binding mechanism reported here may also serve as a basis for the development of bioinspired glues that stick under water and outperform traditional adhesives. ■

"The extreme mechanical stability of SdrG explains... how staphylococci colonize biomaterials so efficiently..."

REFERENCES

1. J. W. Costerton, P. S. Stewart, E. P. Greenberg, *Science* **284**, 1318 (1999).
2. L. F. Milles, K. Schulten, H. E. Gaub, R. C. Bernardi, *Science* **359**, 1527 (2018).
3. T. J. Foster, J. A. Geoghegan, V. K. Ganesh, M. Höök, *Nat. Rev. Microbiol.* **12**, 49 (2014).
4. K. C. Neuman, A. Nagy, *Nat. Methods* **5**, 491 (2008).
5. J. Xiao, Y. F. Dufrêne, *Nat. Microbiol.* **1**, 16186 (2016).
6. K. Ponnuraj *et al.*, *Cell* **115**, 217 (2003).
7. P. Herman *et al.*, *Mol. Microbiol.* **93**, 356 (2014).
8. M. Grandbois, M. Beyer, M. Rief, H. Clausen-Schaumann, H. E. Gaub, *Science* **283**, 1727 (1999).
9. A. Persat *et al.*, *Cell* **161**, 988 (2015).
10. E. V. Sokurenko, V. Vogel, W. E. Thomas, *Cell Host Microbe* **4**, 314 (2008).
11. P. Vitry *et al.*, *mBio* **8**, e01748 (2017).
12. C. Feuillie *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **114**, 3738 (2017).

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NEUROIMMUNOLOGY

Neuronal–immune system cross-talk in homeostasis

Interactions between immune and neuronal cells are pillars in tissue homeostasis

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Maintenance of mammalian tissue homeostasis and function requires coordinated actions of multiple cellular and molecular networks. This complexity is reflected in the immune system, which is composed of a plethora of cells that constitute the innate and adaptive immune system and which can sense multiple endogenous and exogenous factors. Similarly, the nervous system includes a myriad of distinct neurons that perceive, integrate, and respond to ever-changing environmental conditions. Functional interactions between the neuronal and immune systems have been reported in health and disease, such as in multiple sclerosis, autism, cancer, and chronic inflammatory disorders (*1*). More recently, a number of studies have revealed that discrete neuronal and immune cells share anatomical localization and interact functionally, forming neuroimmune cell units (NICUs) that orchestrate tissue homeostasis and integrity (*2*). These findings are provoking a fundamental paradigm shift in our understanding of neuronal–immune cell interactions. A recent noteworthy example is the finding that the nervous system can have a major regulatory effect on multiple innate immune cells with functional impact in several physiological processes (*3*–*8*).

Earlier studies established that signals from the parasympathetic vagus nerve, which connects the brainstem with peripheral organs, can have an anti-inflammatory effect via tuning the activity of macrophages, innate immune cells that engulf pathogens and cell debris, leading to the production of macrophage-derived immunomodulatory molecules (*9*). Bidirectional neuronal-macrophage interactions were also shown to regulate important aspects of intestinal physiology. Notably, intestinal macrophages control myenteric neuron activity and small intestine peristalsis (muscular contractions that move food down the intestine) in response to microbial signals in the intestines (*3*), whereas intestinal pathogenic bacterial infections activate neurons to produce norepinephrine that induces a tissue-protective program in enteric macrophages (*4*). Notably, neuron-associated macrophages are also present in adipose tissue and were shown to buffer sympathetic neuronal activity and fat tissue physiology, thus controlling obesity and organismal metabolism (*10*). Dendritic cells and mast cells (both components of the innate immune system) also interact with peripheral neurons (*1*). For example, upon chemical irritation or infection with fungi, sensory neurons in the skin instruct dermal dendritic cells to produce the cytokine interleukin-23 (IL-23), which activates adaptive T lymphocytes to produce pro-inflammatory cytokines (*11*). Reciprocally, lymphocyte-derived type 2 cytokines—such as IL-4, IL-5, and IL-13—were also shown to induce chronic itch via sensory neuron activation (*12*). Together, these findings demonstrate that neurons can trigger functional molecular cascades that lead to the activation of innate and adaptive immune cells, influencing immunity to infection, chronic inflammation, and restoration of tissue homeostasis. Nevertheless, defining additional pathways that operate in the opposing direction, whereby immune cells can modulate neuronal activity, requires further study.

But how widespread and biologically important is this neuronal-immune interaction? Over the past decade, we have witnessed the formal discovery of innate lymphoid cells (ILCs) and their roles in development, infection, inflammation, metabolic disease, and cancer (*13*). ILCs are a relatively rare cell type, but they are particularly abundant at barrier surfaces that are exposed to the external environment, which are also densely populated with neuronal cells. Group 2 ILCs (ILC2s) are associated with allergy and parasitic worm infections and were reported to respond to vasoactive intestinal peptide signals that were presumably derived from neuronal cells (*14*), suggesting that neuronal-

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