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# Stress-Induced Catch-Bonds to Enhance Bacterial Adhesion

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**Physical forces have a profound influence on bacterial cell physiology and disease. A striking example is the formation of catch-bonds that reinforce under mechanical stress. While mannose-binding by the *Escherichia coli* FimH adhesin has long been the only thoroughly studied microbial catch-bond, it has recently become clear that proteins from other species, such as staphylococci, are also engaged in such stress-dependent interactions.**

## Mechanical Cues Influence Cellular Adhesion

Mechanical forces play an essential role in eukaryotic and prokaryotic cell behavior, physiology, and disease [1]. During adhesion, cells experience hydrodynamic forces that they need to cope with and take advantage of to fulfill their functions. For effective adhesion to host cells and extracellular matrix components, the first step leading to invasion and subsequent dissemination, bacterial pathogens need to overcome and adjust to shear forces encountered, for example, in blood vessels or respiratory tracts. To do so, they use a plethora of surface adhesion proteins called adhesins. Flow-chamber assays have revealed that bacterial adhesion can be enhanced by shear, as exemplified by the clumping of *E. coli* cells and erythrocytes through dedicated pili adhesins [2].

## Catch-Bonds

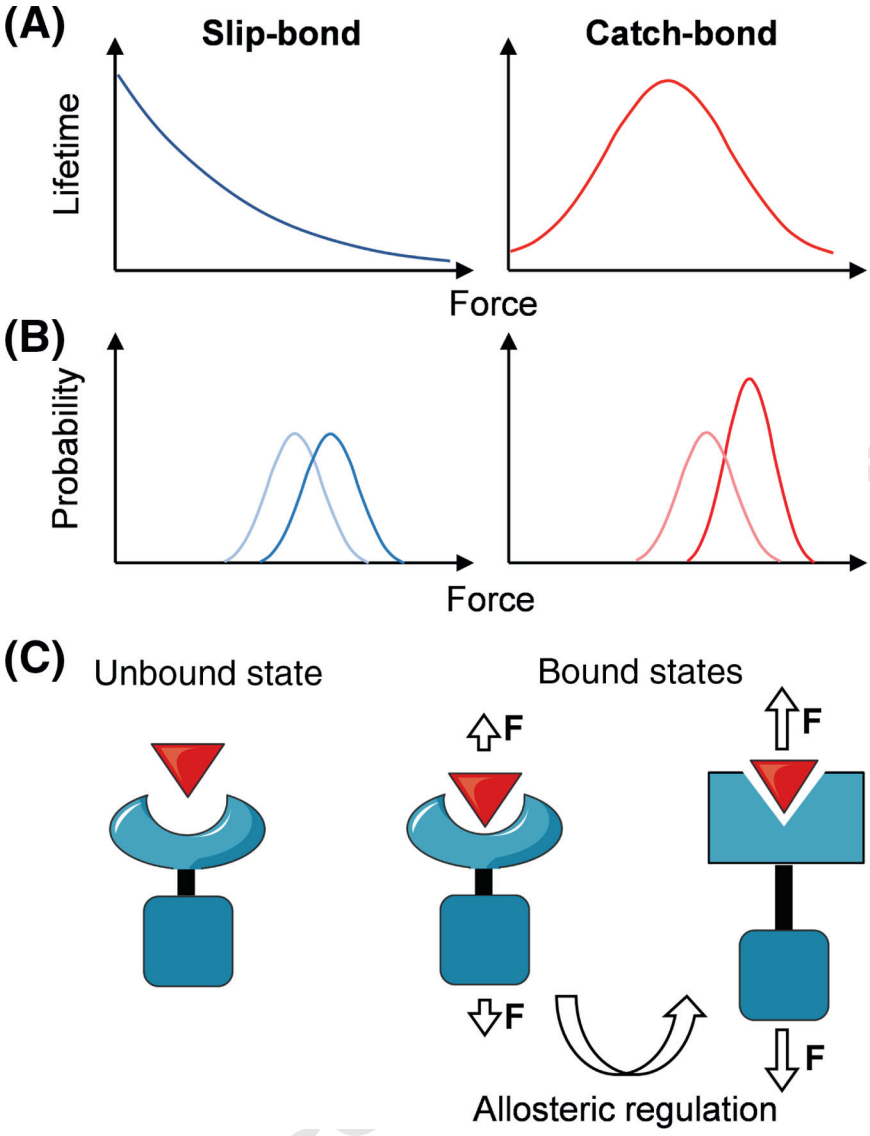
What is the molecular mechanism driving shear-enhanced bacterial adhesion? It is commonly accepted that receptor–ligand interactions slip apart more easily under increasing external force. This originates from the exponential decrease of the lifetime of the adhesion complex bond (called the 'slip-bond'), thus favoring the detachment of cells from their hosts under increasing shear. However, it had been initially hypothesized, and later demonstrated, that mechanical stimulation can actually promote cell adhesion. Subsequently, a theoretical distinction was made between slip-bonds that dissociate faster under load, and catch-bonds whose lifetime increases with force in a conceptually similar manner to a Chinese finger trap, until a critical force threshold is reached where they eventually break in a slip-fashion (Figure 1A,B) [3].

The first direct proof of catch-bonds came in 2002 with the binding of *E. coli* FimH to mannose residues [2]. Thomas *et al.* demonstrated that a structural change in the adhesin under mechanical tension was responsible for shear-enhanced bacterial adhesion (see details in the following section). Focusing on eukaryotes, Marshall *et al.* demonstrated the catch-bond formed by the P-selectin receptor binding to its ligand PSGL-1, by means of single-molecule experiments [4]. This behavior explains how leucocytes could switch from a rolling adhesion at low shear to a steady attachment at high shear to endothelial cells [5].

## Catch-Bonds in Bacterial Virulence

While catch-bonds have been suggested to potentially occur in various bacterial pathogens, currently only one microbial catch-bond has been thoroughly characterized and structurally solved at the molecular level, that is, the complex formed between FimH adhesins on uropathogenic *E. coli* and mannose residues on epithelial cells [6,7]. FimH consists of a lectin domain, where the binding pocket for mannose is located, and a pilin domain integrating FimH into the fimbriae. The anchoring pilin domain acts as an autoinhibitor [8], favoring a loosened conformation of the binding pocket, which is, in turn, indicative of the inactive low-affinity state of the adhesin. Importantly, this pilin domain is connected to the lectin domain by a linker chain. When the FimH adhesin is subjected to tensile load, the pilin and lectin domains are stretched apart through the extension of the linker chain that leads to the untwisting of the β sandwich fold of the lectin domain. This subsequently favors a tight high-affinity conformation of the distally located mannose-binding pocket (Figure 1C). These allosteric properties of FimH provide a structural explanation of the shear-enhanced catch-bond behavior between FimH and mannose [9]. Yakovenko *et al.* indeed demonstrated that the lifetime of those bonds increases along with the external load between 60 and 100 pN, typical of physiological shear stresses. Other catch-bond models were proposed later, including the deformation and the sliding–rebinding models that still remain to be experimentally proven [10], though for some systems, like P-selectin adhesion, such models have already been numerically validated [11]. In the deformation model, tensile force directly induces a structural change of the receptor binding pocket and/or ligand, resulting in a tighter fit of both. In the sliding–rebinding model, force leads to alignment of the binding pocket and the ligand in such a way that new transient contacts occur and facilitate rebinding.

In recent years, single-molecule experiments have revealed that staphylococcal adhesins, such as SdrG, ClfA, and ClfB, bind to their host proteins with ultrastrong forces, similar to that of a covalent bond (in the nN range), while showing moderate affinity towards their protein ligands (see e.g., [12]). These adhesins bind some of their ligands by the multistep dock, lock,




Q2 Figure 1. Force-Enhanced Adhesion. (A) Dependence of the lifetime of receptor–ligand interactions on the force load for slip- and catch-bonds, in constant load single-molecule experiments. (B) Probability distribution of adhesive bond rupture as a function of force for low (light colors) and high (dark colors) loading rates for slip- and catch-bonds, in constant velocity single-molecule experiments. (C) Schematic of the allosteric model Q1 proposed for catch-bond formation in the bacterial FimH [6]. Tensile force, through the extension of a linker look between FimH domains, induces a conformational change in the lectin domain switching from a low-ligand binding affinity to a high-ligand binding affinity state.

and latch (DLL) mechanism that relies on dynamic conformational changes of the adhesin-binding pocket upon insertion of the ligand, as exemplified by the prototypical Q7 *Staphylococcus epidermidis* SdrG–Fg (fibrinogen) bond [13]. As uncovered by molecular dynamics simulations, the target peptide confined in a screw-like manner in the binding pocket of SdrG requires the simultaneous rupture of numerous hydrogen bonds in a cooperative shear geometry, resulting in an extreme mechanical strength and highly stable complexes [13]. Remarkably, atomic force microscopy (AFM) studies have unraveled the force-strengthening of some adhesin–ligand complexes involved in DLL with increasing load. Such behavior enables staphylococci to attach firmly to their targets, while resisting mechanical stresses associated with fluid flow and surface contacts [14]. Together with flow-chamber assays, these experiments point to a catch-bond mechanism, even though a direct demonstration for this is still missing.

## Direct Demonstration of a Catch-Bond in a Gram-Positive Pathogen

A recent study from our group directly and unambiguously identified and characterized the first catch-bond mechanism in a Gram-positive pathogen using single-molecule force-clamp spectroscopy [15]. We found that the DLL interaction between staphylococcal surface protein SpsD and Fg is extremely strong (rupture force of ~2 nN) and exhibits an unusual catch–slip transition (Figure 2A). The bond lifetime first grows with increasing force, but ultimately ceases to behave as a slip-bond beyond a critical force orders of magnitude higher than previously investigated on purified complexes (~1100 pN). Conventional catch bond models, based on an entropic regime, where transition between bound, transient, and unbound states occur, failed to explain such observations. Rather, it was proposed that the DLL catch-bond involves the force-induced formation of long-lived hydrogen bonds between the ligand and the adhesin within the binding pocket, which, in turn, critically depends on force propagation and shear geometry [14].

## Concluding Remarks and Future Perspectives

Catch-bonds represent a competitive advantage to help microbial cells tune their adhesion depending on flow conditions, that is, by binding loosely and spreading under low flow and resisting shear and remaining firmly attached under high flow. Notably, the catch-binding phase allows

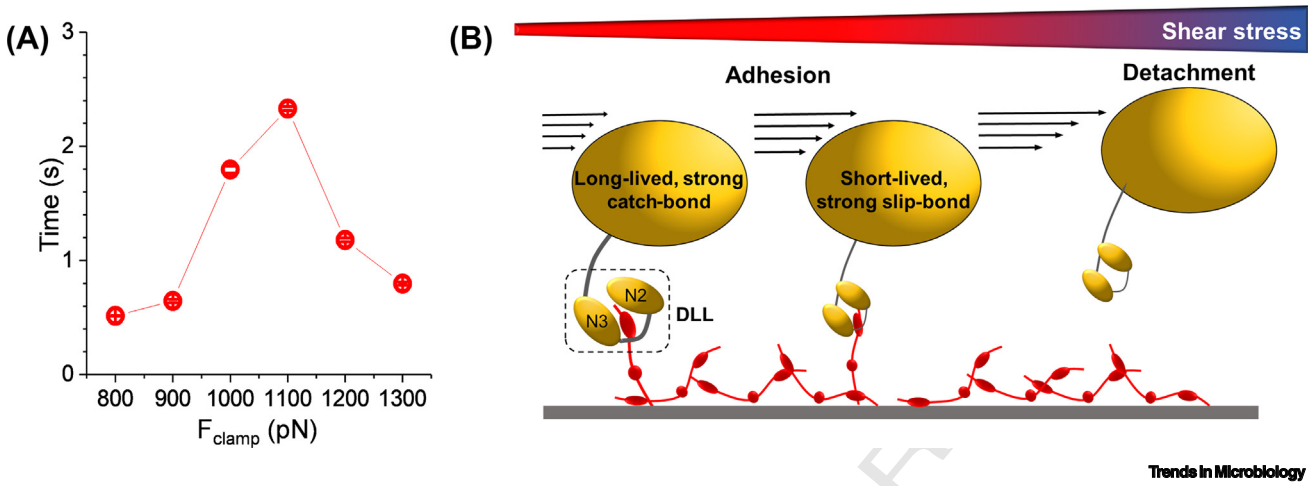



Figure 2. Demonstration of a Catch-Bond in a Staphylococcal Adhesin. (A) Dependence of the bond lifetime of *Staphylococcus pseudintermedius* SpsD interacting with fibrinogen (Fg) on the force load ($F_{clamp}$) showing a critical force threshold beyond which a catch-bond behavior is observed. (B) Schematic of bacterial behavior when facing an Fg-coated surface under shear stress. The dock, lock, and latch (DLL) interaction between SpsD and Fg occurs, through the N2 and N3 subdomains of the adhesin: Fg (in red) inserts in the trench formed by these subdomains driving a conformational change at the C terminus of the N3 domain, which further leads to the lock of the peptide in a screw-like manner. The DLL allows strong and long-lived bonds through a catch-bond mechanism, enabling a firm attachment to the target. At sufficient stress, these bonds are overpowered and subsequently favor the detachment of bacteria and their potential dissemination to other colonization sites.

bacteria to strengthen colonization and biofilm formation under shear stress, whereas the slip-binding phase, which persists at even higher shear, would help detachment, dissemination, and colonization of new sites (Figure 2B). In the future, we are confident that the advent of single-molecule assays will uncover new catch-bond behaviors. Recently, Liu *et al.* unraveled how gut microbes regulate cell adhesion at high shear through the mechanically stable Doc:Coh complex, allosterically regulated by a neighboring domain which induces what they call a catch-bonding mechanism, though missing lifetime measurements [16]. Hence, catch-bonds might be much more widespread among microbes than initially thought. Unraveling the molecular mechanisms and structural insights of catch-bond behavior would pave the way for the development of antiadhesive therapies that could inhibit such shear-dependent bacterial adhesion. This would result in promising alternatives to conventional antibiotics that are incapable of treating the increasing incidence of multidrug-resistant bacterial infections.

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