NANO LETTERS

pubs.acs.org/NanoLett

Letter

Catch Bond-Mediated Adhesion Drives *Staphylococcus aureus* Host Cell Invasion

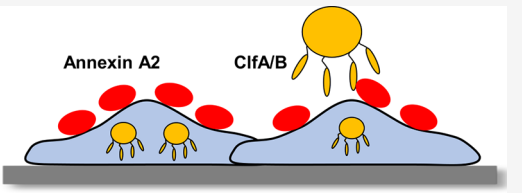
Can Wang, Telmo O. Paiva, Chiara Motta, Pietro Speziale,* Giampiero Pietrocola,* and Yves F. Dufrêne*

Cite This: *Nano Lett.* 2023, 23, 5297−5306

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: Various viruses and pathogenic bacteria interact with annexin A2 to invade mammalian cells. Here, we show that *Staphylococcus aureus* engages in extremely strong catch bonds for host cell invasion. By means of single-molecule atomic force microscopy, we find that bacterial surface-located clumping factors bind annexin A2 with extraordinary strength, indicating that these bonds are extremely resilient to mechanical tension. By determining the lifetimes of the complexes under increasing mechanical stress, we demonstrate that the adhesins form catch bonds with their ligand that are capable to sustain forces of 1500−1700 pN. The force-dependent adhesion mechanism identified here provides a molecular framework to explain how *S. aureus* pathogens tightly attach to host cells during invasion and shows promise for the design of new therapeutics against intracellular *S. aureus*.



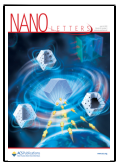
KEYWORDS: *single-molecule experiments, Staphylococcus aureus, clumping factors, annexin A2, host invasion, catch bonds*

Annexins are a group of human proteins that play multiple functional roles, such as controlling membrane scaffolding, promoting trafficking and vesicle organization, mediating cell−cell interactions and, in the extracellular environment, inducing fibrinolysis, coagulation, inflammation, and apoptosis.[1−3] An important member of the family is annexin A2, which is found in endothelial cells, epithelial cells, monocytes, macrophages, and most cancer cells. While annexin A2 is mostly present in the cytoplasm, it can also be found associated with the plasma membrane. This protein is composed of a variable "head" at the N-terminus and of a highly conserved "core" domain located at the C-terminus[4] (Figure 1a). The N-terminal domain is a variable, intrinsically disordered region that contains sites for post-translation modifications including acetylation, serine/tyrosine phosphorylation, and for binding to S100A10, a protein that forms heterotetramers with annexin A2. The conserved C-terminal core domain is composed of four homologous repeats of about 70 amino acids, each consisting of a right-handed superhelix of five α-helices.[5] The core domain interacts with calcium[6] and with many biomolecules, including RNA,[7−9] phospholipids,[10−12] heparin,[13] and F-actin.[10,14]

Viruses[15] and pathogenic bacteria[16] interact with surface-exposed annexin A2 to favor colonization and invasion of host cells. Annexin A2 bridges cytomegaloviruses to cell membranes to enhance infection[17−19] and binds to human papilloma virus L2 capsid protein[20,21] and Enterovirus type 71 for host cell entry.[22−24] Annexin A2 colocalizes with spike glycoprotein S2 protein of SARS CoV-2 suggesting a potential role of S2 protein in viral invasion.[25] Intracellular annexin A2 is targeted by the nonstructural protein 1 involved in the replication and pathogenesis of avian influenza virus.[26] Annexin A2 exposed on mammalian cells serves as a specific ligand for *Pseudomonas aeruginosa*, leading to its internalization.[27] Together with cellular proteins NHERF2, it forms a scaffold complex that links adjacent Tir molecules during attachment of enteropathogenic and enterohemorrhagic *Escherichia coli* to host cells.[28] The ligand also mediates specific binding to uropathogenic *E. coli*,[29] *Salmonella typhymurium*,[30] and *rickettsiae*,[31] and the community-acquired respiratory distress syndrome toxin mediates annexin A2-dependent *Mycoplasma pneumoniae* interaction with eukaryotic cells and determines disseminated inflammation and tissue pathologies.[32]

There is also compelling evidence that Gram-positive bacteria use annexin A2 for adherence and invasion. Pneumococcal surface protein K expressed by uncapsulated *Streptococcus pneumoniae* mediates bacterial adherence to epithelial cells via direct interaction with annexin A2.[33] *S. pneumoniae* adhesin A is involved in the annexin A2-dependent attachment and colonization of nasopharyngeal epithelial cells.[34] Western blotting, ELISA assays, and pull-down experiments showed that recombinant *Staphylococcus aureus* clumping factor A (ClfA; Figure 1b) interacts with annexin A2.[35] ClfA and

Received: April 13, 2023
Revised: May 20, 2023
Published: June 2, 2023



https://doi.org/10.1021/acs.nanolett.3c01387
Nano Lett. 2023, 23, 5297−5306

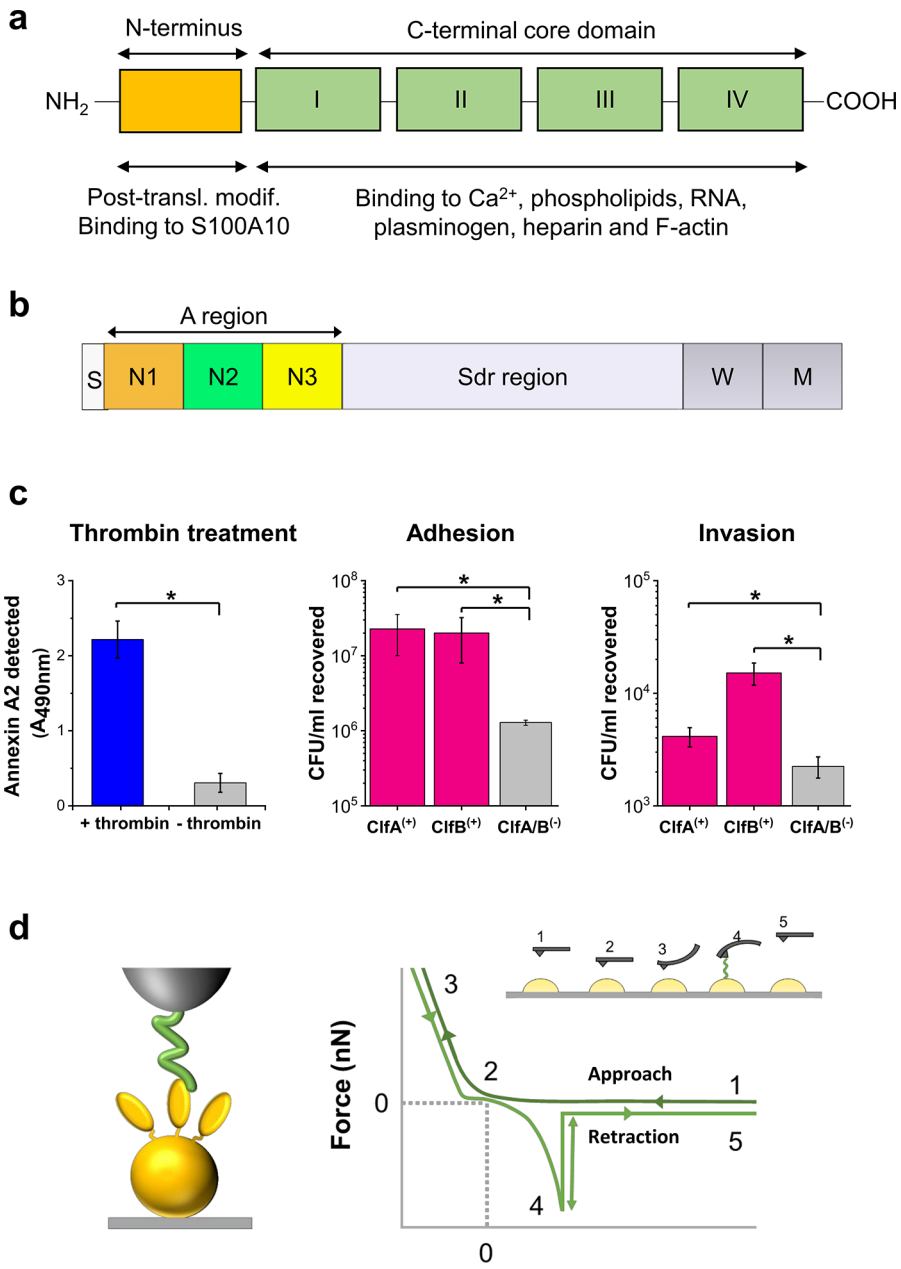


Figure 1. Studying the binding of annexin A2 by clumping factors A and B. (a) Annexin A2 (339 residues) is composed of two major domains, the amino-terminal domain or head region and the carboxyl-terminal core domain. The amino-terminal tail region is the site for post-translational modification and encompasses the redox reactive cysteine residues (Cys-8) and the nuclear export sequence (Val-3-Leu-12) and an amphipathic α-helix which binds to the S100A10 protein. The carboxyl-terminal core domain encompasses four predominantly α-helical repeatx each containing 70 amino acids. Each repeat segment contains five α-helices in which four helices are oriented antiparallel and the fifth one is lying perpendicular to the other helices. The carboxyl-terminus core domain contains binding sites for heparin and RNA, calcium and phospholipids, plasminogen, and as well as for F-actin. The binding sites for viruses and bacteria remain to be determined. (b) Schematic diagram of the domain organization of ClfA (896 amino acid residues) and ClfB (933 residues) showing the ligand-binding A-region made of the N1N2N3 subdomains, the serine-aspartate repeat region (Sdr), the wall-spanning domain (W), and the membrane anchor (M). (c) Annexin A2 exposure on thrombin-treated or -untreated HUVEC cell monolayers was detected incubating the wells with an annexin A2 antibody (left). Host cell adhesion (center) and invasion (right) were tested by incubating *S. aureus* cells with thrombin-treated HUVEC cell monolayers. Inocula, adherent, and internalized bacteria were quantified by viable counting. Error bars show s.d. of the means from at least three determinations performed in triplicate (Student's test: *, $P < 0.05$). (d) The strength and dynamics of single adhesin−ligand interactions were studied by single-molecule force spectroscopy (SMFS) in which annexin A2-modified tips are used to register force−distance curves on *S. aureus* bacteria expressing exclusively ClfA or ClfB.

annexin A2 partially colocalize on the host cell plasma membrane, but essentially concentrate in the cytosol, indicating ClfA cellular uptake.[35] These *in vitro* observations suggest that ClfA binds to annexin A2 and that this interaction plays a role in *S. aureus* invasion. Along the same line, *S. aureus* has recently been shown to use ClfB to bind annexin A2 for internalization by epithelial cells. Bacterial-associated annexin induced F-actin rearrangement and ClfB was identified in annexin-precipitated proteins from *S. aureus* lysates.[36] Pretreatment with recombinant ClfB triggered *S. aureus* internalization

https://doi.org/10.1021/acs.nanolett.3c01387
Nano Lett. 2023, 23, 5297−5306

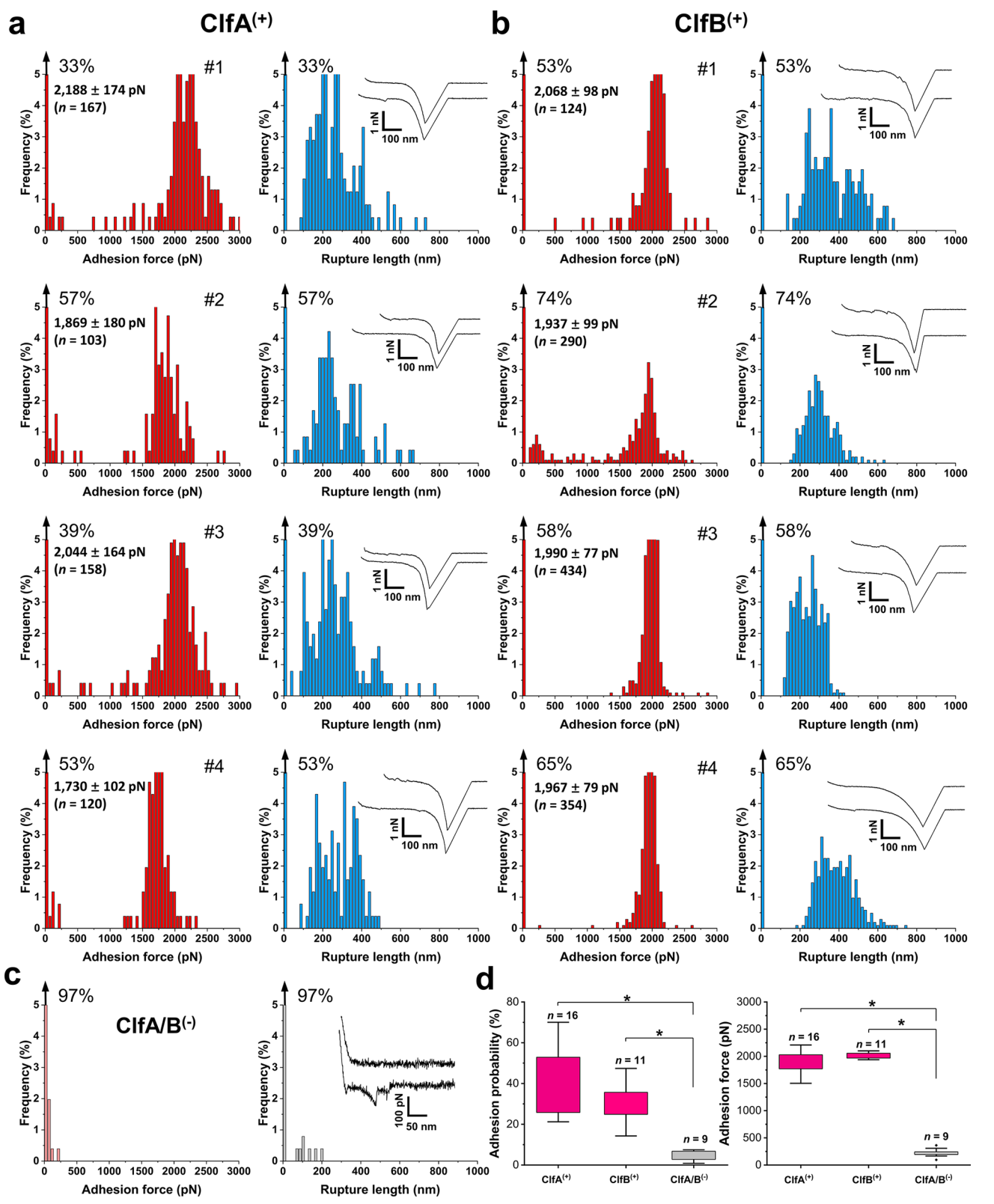


Figure 2. Strength of single clumping factor–annexin A2 interactions in living *S. aureus* bacteria. (a, b) Adhesion force (left) and rupture length (right) histograms obtained by recording force–distance curves in PBS between four different *S. aureus* ClfA$^{(+)}$ and ClfB$^{(+)}$ cells and AFM tips functionalized with annexin A2, at a retraction velocity of 1 μm s^{-1}. Insets show representative adhesive force profiles. (c) Representative force data obtained with *S. aureus* lacking ClfA and ClfB. (d) Box plot of adhesion probability (left) and adhesion force (right) for ClfA$^{(+)}$ cells (n = 16 cells from 3 independent cultures), ClfB$^{(+)}$ cells (n = 11 cells from 6 independent cultures), and ClfA/B$^{(-)}$ cells (n = 9 cells from 2 independent cultures). P values were determined by Mann–Whitney test. *, $P < 0.05$ was considered as significant. If present, the arrow at the top left of a histogram stands for the nonadhesive events.

https://doi.org/10.1021/acs.nanolett.3c01387
Nano Lett. 2023, 23, 5297–5306

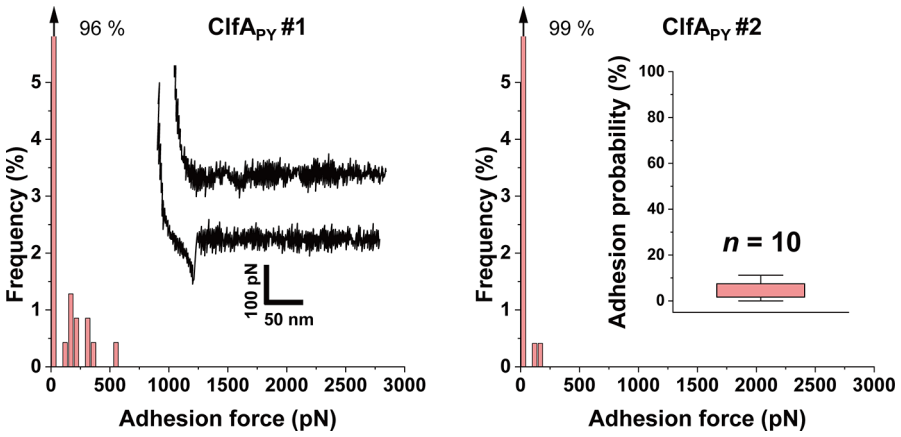


Figure 3. Substitutions within the ClfA N2N3 binding trench show that ClfA and annexin A2 engage in a dock lock and latch interaction. Representative adhesion force histograms with force curves (left inset) obtained in PBS between annexin-tips and two cells from the *S. aureus* ClfA$_{PY}$ strain expressing ClfA carrying amino acid substitutions within the N2N3 binding trench that prevent DLL ligand-binding. Box plot (right inset) showing that the adhesion probability of ClfA$_{PY}$ (n = 10 cells from 3 independent bacterial cultures) is dramatically reduced compared to the ClfA$^{(+)}$ and ClfB$^{(+)}$ strains.

and the action of the adhesin was specifically blocked by annexin antibodies.[36] While annexin A2-binding by Clf proteins appears to be important for host cell adhesion and invasion, the molecular details of this interaction are unknown. Here, we use single-molecule experiments on living bacteria to demonstrate that ClfA and ClfB form very high strength catch bonds with annexin A2, and that this force-enhanced adhesion is a key mechanism in *S. aureus* host cell invasion.

We initially tested the ability of ClfA and ClfB to bind surface-exposed annexin A2 for adhesion and invasion. Bacterial cells from the *S. aureus* SH1000 strain expressing either ClfA or ClfB (hereafter called ClfA$^{(+)}$ and ClfB$^{(+)}$ cells) but not the adhesins FnBPA and FnBPB, were incubated with HUVEC monolayers treated with thrombin as this molecule triggers a much higher exposure of annexin A2 on the plasma membrane (Figure 1c, left).[37] Hence, experimental conditions were used where the impact of adhesins such as FnBPA and B is significantly reduced and the exposure of the Clf ligand (annexin A2) optimized. Both ClfA$^{(+)}$ and ClfB$^{(+)}$ bacteria attached to HUVEC cells in large amounts, while attachment of bacteria lacking both adhesins was substantially lower (Figure 1c, center). In internalization assays (Figure 1c, right), we found that ClfA$^{(+)}$ and ClfB$^{(+)}$ bacteria invaded at substantial levels, with a more pronounced invasion for ClfB$^{(+)}$ ones. These observations show that annexin A2 binding by ClfA and ClfB is important for *S. aureus* adhesion and invasion.

We sought to characterize the mechanical strength of the interactions of ClfA and ClfB with annexin A2 using single molecule force spectroscopy (SMFS; Figure 1d). Force–distance curves were registered between AFM tips functionalized with annexin A2 and living *S. aureus* bacteria. In Figure 2a and b we present the adhesion forces and rupture distances obtained for representative ClfA$^{(+)}$ and ClfB$^{(+)}$ cells (including cells from independent cultures; for data on more cells, see Figure 2d, Figure S1). Most curves featured well-defined, extremely strong adhesion forces, with a mean of 2005 ± 120 pN for ClfA (mean ± s.d., from a total of n = 1978 adhesive curves from 11 cells) and 1932 ± 160 pN for ClfB (n = 1604 adhesive curves from 16 cells). While the magnitude of strong forces slightly varied from one cell to another, their distributions were always narrow, implying they originate from the rupture of single protein complexes. A multimodal force distribution is expected when a variable number of bonds break simultaneously, which was not observed here. Strong bonds ruptured at ∼200–400 nm, reflecting extension of the intrinsically disordered C-terminal stalk domains of the adhesins (residues 543–896 in ClfA and 541–874 in ClfB) and of annexin (346 residues). The mechanically ultrastable A domains of Clfs are unlikely to unfold. Of note, adhesion force maps (Figure S2) documented heterogeneous distributions of ClfA and ClfB at the bacterial cell surfaces, with the formation of nanoclusters.

Several observations support the view that strong forces result from specific dock lock and latch (DLL) interactions: (i) ∼2 nN forces were also observed with tips modified using an NHS surface chemistry (Figure S3) rather than a PEG-benzaldehyde linker; (ii) strong adhesion was missing on cells lacking ClfA and ClfB (Figure 2c, Figure S4); (iii) strong forces are in the range of that reported for prototypical DLL interactions;[38−42] (iv) binding was essentially abolished when using an *S. aureus* strain (ClfA$_{PY}$) expressing ClfA carrying substitutions in key amino acids (specifically, Pro 336 and Tyr 338, both located in the N2 subdomain and concurring to the hydrophobic environment of the trench) involved in DLL binding (Figure 3, Figure S5).[41]

We next measured the strength of the Clf-annexin A2 bonds (F) while varying the rate at which force is applied (loading rate, LR, assessed from the force vs time curves). Figure 4a,b shows the dynamic force spectroscopy (DFS) data registered with retraction speeds ranging from 0.5 μm s^{-1} to 10 μm s^{-1} (data pooled from 9388 and 5312 adhesive curves on six ClfA$^{(+)}$ and five ClfB$^{(+)}$ cells, respectively). In line with the Bell–Evans (BE) theory,[43] a semilog relationship was observed between the most-probable forces and the LRs (Figures 4a,b), yielding a position of the energy barrier that separates the bound from the unbound state of x_u = 0.063 and x_u = 0.077 nm, and an off-rate constant at thermal equilibrium of k_{off}^0 = 3.49 × 10^{-12} s^{-1} and k_{off}^0 = 1.27 × 10^{-14} s^{-1}, for ClfA and ClfB respectively. The very low k_{off}^0 values illustrate the high lifetime and mechanostability of the complexes.

The above results show that both adhesins feature similar kinetic parameters for the high forces. However, ClfA, but not ClfB, exhibited weaker intermediate forces, in the 1200–1700

https://doi.org/10.1021/acs.nanolett.3c01387
Nano Lett. 2023, 23, 5297−5306

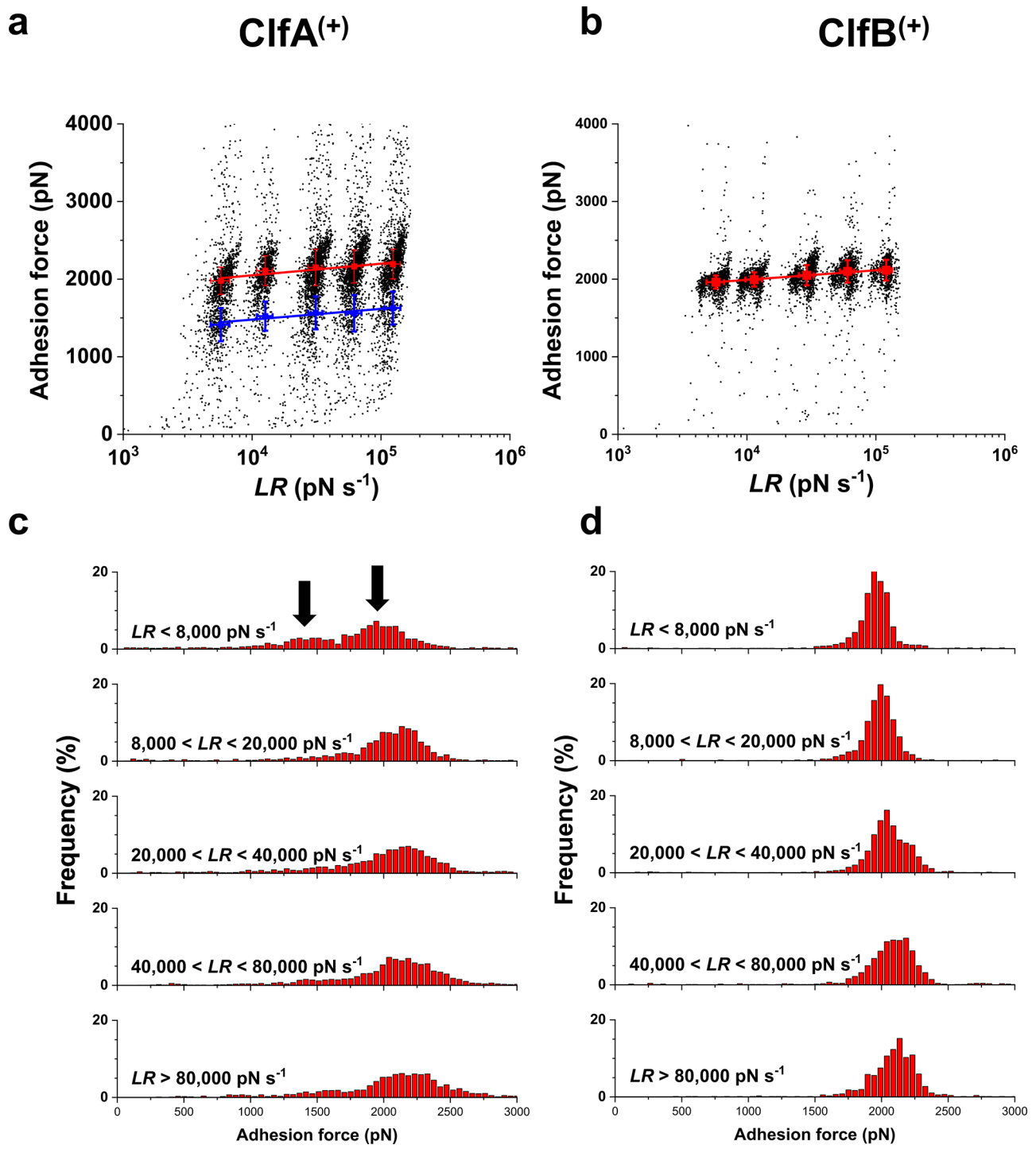


Figure 4. Dynamic force spectroscopy of the clumping factor-annexin A2 interactions. (a, b) DFS plots showing the distribution of the rupture forces vs loading rates between annexin A2-tip and ClfA$^{(+)}$ (a) and ClfB$^{(+)}$ cells (b). For ClfA$^{(+)}$ cells, data were pooled from n = 9388 force curves from 6 cells from 4 independent bacterial cultures; ClfB$^{(+)}$ cells, from n = 5312 force curves from 5 cells from 3 independent bacterial cultures. The red lines represent Bell−Evans model fit of the data, showing the expected loading-rate dependency. x_u = 0.063 nm and $k_{off}^0 = 3.49 \times 10^{-12}$ s^{-1}, x_u = 0.077 nm and $k_{off}^0 = 1.27 \times 10^{-14}$ s^{-1}, for ClfA and ClfB were extracted, respectively. For ClfA$^{(+)}$, a second fit at lower forces is shown (blue), with x_u = 0.068 nm and $k_{off}^0 = 3.47 \times 10^{-9}$ s^{-1}. Error bar indicates s.d. of the mean value. (c, d) Histograms of force distribution for discrete ranges of *LRs*, documenting an unusual bimodal force distribution for ClfA$^{(+)}$ (arrows).

pN range, that could also be described by the BE model, with x_u = 0.068 nm and $k_{off}^0 = 3.47 \times 10^{-9}$ s^{-1}, values indicating a lower bond lifetime. Sorting adhesion forces by discrete ranges over *LR* clearly revealed the occurrence of a bimodal force distribution for ClfA (Figure 4c). Earlier studies have shown that besides the Fg γ-peptide N2N3 binding trench of ClfA, there is a second binding site located at the top of the N3 domain which is critical for an overall high affinity Fg-ClfA interaction.[38,44] One could therefore argue that the intermediate forces are associated with a second distinct binding site on the adhesin. However, the lack of adhesion forces larger than ∼500 pN observed with the *S. aureus* ClfA$_{PY}$ strain

https://doi.org/10.1021/acs.nanolett.3c01387
Nano Lett. 2023, 23, 5297−5306

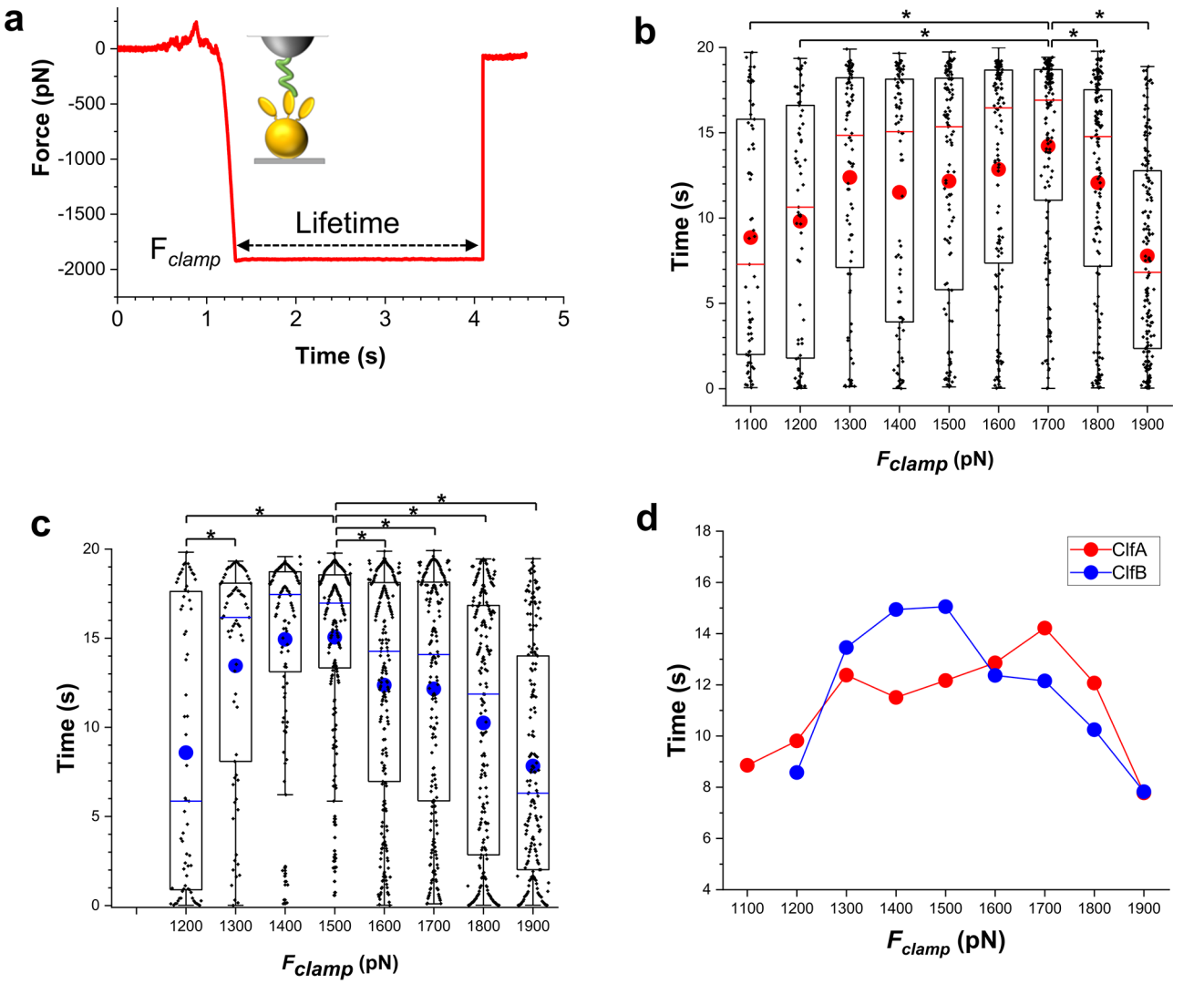


Figure 5. Clumping factor–annexin A2 interactions feature catch-slip bond transitions. (a) Representative force–clamp curve (force vs time). The bond lifetime is given by the constant force regime indicated by black dots arrow. (b, c) Box plots showing the lifetime data obtained from force vs time for ClfA (b) and ClfB (c). ClfA, data pooled from n = 71, 71, 88, 87, 125, 140, 150, 154, and 167 curves for F_{clamp} = 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, and 1900 pN, respectively, recorded on a total of 6 cells from 4 independent cultures. ClfB, data pooled from n = 67, 73, 112, 216, 225, 205, 219, and 186 curves for F_{clamp} = 1200, 1300, 1400, 1500, 1600, 1700, 1800, and 1900 pN, respectively, recorded on a total of 5 cells from 5 independent cultures. Circles are the mean values, lines the median, boxes the 25–75% quartiles, and whiskers the 10–90% interval. Difference of distributions were determined using Dunn-Sidak multiple comparison test. *, $P < 0.05$. (d) Bond lifetimes measured at different F_{clamp} showing the catch-slip transition for ClfA and ClfB.

(Figure 3) allows us to rule out this possibility. Rather, we argue that the intermediate forces reflect the rupture of DLL complexes that are not tightly stabilized due to metastable geometries.

The high lifetimes of the ClfA and ClfB interactions estimated from the DFS data are in contrast with the moderate lifetimes obtained from bulk affinity experiments,[13,45−47] thus indicating that unbinding occurs through a different pathway under tensile loading. We hypothesized that the force-dependent pathway may result from the formation of long-lived catch bonds, as observed for SdrG and SpsD DLL interactions.[48,49] To test this, we used force-clamp spectroscopy (Figure 5), where the adhesin–ligand bond was clamped at forces ranging from 1100 pN to 1900 pN, while recording the time that the adhesin and ligand stay in contact before the bond ruptures spontaneously. The bond lifetime (τ) was assessed from the persistent time extracted from the force vs time curves (Figure 5a).

For ClfA (Figure 5b), mean lifetimes of 8.9, 9.8, 12.4, 11.5, 12.2, 12.9, 14.2, 12.1, and 7.8 s were obtained at clamping forces of 1100, 1200, 1300, 1400, 1500, 1600, 1700, 1800, and 1900 pN, respectively. For ClfB (Figure 5c), the lifetimes were 8.6, 13.5, 14.9, 15.1, 12.4, 12.2, 10.2, and 7.8 s at 1200, 1300, 1400, 1500, 1600, 1700, 1800, and 1900 pN. Hence, an increase in lifetimes was observed in the 1100–1700 pN and 1200–1500 pN force ranges for ClfA and ClfB (Figure 5d) reflecting a catch bond behavior. Beyond these critical forces, the lifetimes switched back to lower values, as expected for slip bonds. Intriguingly, ClfA exhibited a catch-slip transition more complex than ClfB (Figure 5d), thus correlating with the DFS plots and indicating that the ClfA–annexin A2 interaction is indeed characterized by both intermediate and high forces. Lastly, we note that survival plots (Figure S6), i.e., number of

https://doi.org/10.1021/acs.nanolett.3c01387
Nano Lett. 2023, 23, 5297−5306

intact bonds versus time for the different F_{clamp} values, showed that the survival probability decreased linearly (in a semilog representation) for times smaller than ∼10 s, after which the probability dropped nonlinearly.

Collectively, our results demonstrate high force catch bonds between ClfA/B adhesins and annexin A2. A variety of bacterial pathogens, including *P. aeruginosa*, *E. coli*, *S. typhymurium*, *rickettsiae*, and *S. aureus*, interact with annexin A2 on the surface of mammalian cells,[27,28,30,31,35,36] yet the molecular details of these interactions and their role in host cell invasion have remained largely unexplored. This work represents the first identification of a catch bond mechanism used by a bacterial pathogen for host cell invasion, thus highlighting the role of catch bonding in governing *S. aureus* pathogenicity.

ClfA/B-annexin A2 complexes have a force resilience similar to that of covalent bonds, and in the range of that reported for staphylococcal DLL-interactions.[38−40,42,48,49] Analysis of the $ClfA_{PY}$ mutant strain demonstrates that the ∼2 nN binding forces are associated with a DLL mechanism, in which the adhesin N2N3 binding domains undergo conformational changes under stress, enabling the formation of long-lived bonds with its ligand. Adhesins are generally heterogeneously distributed across the cell surface and localize in nanoclusters that are likely to strengthen bacterial adhesion *in vivo* through multivalent binding.[50]

ClfA/B-annexin A2 interactions exhibit catch−slip bond transitions at critical forces of 1.5−1.7 nN. Below this transition force, the bond is longer-lived with increasing mechanical force (catch-bond behavior) while above it, the bond weakens with further increasing force (slip-bond behavior). The critical force is orders of magnitude larger than that of all other cellular receptor−ligand bonds investigated so far, e.g., FimH, PSGL-1 ligand, actin/myosin, and T cell receptors (transition forces smaller than 10 pN).[51−54] The bond lifetime and critical force of the catch−slip transition are substantially larger than those of the DLL SpsD-Fg interaction,[48,55] which is indicative of a higher mechanical stability. Notably, ClfA, but not ClfB, exhibit both intermediate and high binding forces, indicating that the adhesin forms DLL complexes that are not fully stabilized. Perhaps, this unusual binding behavior explains the lower capacity of ClfA to support host cell invasion.

Lifetime measurements generally follow an exponential decay, which is not observed here. While this deviates from classical catch-bond behaviors, we recently found that in the case of the very strong interaction between the *S. aureus* adhesin SdrE and complement factor H, lifetime values follow exponential distributions for all clamping forces studied, except for those matching the critical force at which the catch−slip transition occurs.[56] We believe this atypical behavior, similar to what we observe in the present study, is related to the unique binding strength and complexity of the mechanisms at play, which are associated with a much higher mechanostability than in typical receptor−ligand bonds. This is supported by the fact that not only the critical forces we measured, but also the bond lifetimes, are much larger than those reported for the DLL interaction between the staphylococcal adhesin SpsD and its Fg ligand.[48] Formation of long-lived catch bonds is of relevance in the early stage of invasion as surface-attached bacteria experience mechanical cues induced by fluid flow and cellular contacts.[57−60] *In vivo*, annexin A2 is exposed to shear stress associated with blood flow under specific physiological conditions, e.g., when coagulation starts and prothrombin is activated into thrombin.

Molecular dynamics (MD) simulations of staphylococcal adhesins engaged in a DLL mechanism[41,42,61,62] have shown that the target ligand peptide is confined in a screw-like manner in the adhesin binding pocket. Rupture of the complex involves the simultaneous breakage of numerous hydrogen bonds in a cooperative shear geometry, yielding an extreme mechanical strength. We argue that the high mechanical stability of the Clf-annexin complexes results from an intricate hydrogen bond network between the ligand peptide backbone and the adhesin. For SdrG, MD simulations revealed that a catch bond mechanism explains the force-enhanced mechanical strength and stability of the complex.[49] While allowing for thermal dissociation in a low-force regime,[63] an entirely different mechanical dissociation path emerged in a high-force regime, revealing a complex mechanism that does not depend on the peptide amino acid sequence.

An interesting question is how do our measured ligand-binding forces compare with the anchoring strengths of the adhesin and annexin proteins? ClfA and ClfB belong to a family of adhesion proteins that are covalently attached to the bacterial cell wall, the Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs), and which target host extracellular proteins.[64] Annexin A2 tightly associates with the host plasma membrane via Ca^{2+}-dependent ionic binding to negative charged phospholipids.[65] Notably, this annexin can bind up to 12 Ca^{2+} ions, suggesting it engages in multivalent interactions with the cell membrane. Other interactions may play a role in membrane association, as some annexins, like annexin A5, bind hydrophobic chains of membrane lipids via insertion of a tryptophan residue into the membrane,[66] while annexin A13 can insert into membranes through the N-terminal myristoylated domain.[67] Therefore, it is possible that similar hydrophobic bonds are involved in binding annexin A2 to the host membrane. Membrane anchoring of annexin A2 could also be reinforced by the formation of heterotetrameric complexes with S100A10 and by interaction with specific proteins and cholesterol-rich lipid raft microdomains.[68] In addition, self-association of the annexin into oligomers results in stable clusters on plasma membranes.[69] These putative interactions are not mutually exclusive, but may cooperate in the stabilization of the annexin A2-membrane association.

Finally, it is tempting to speculate on the mechanism of bacterial internalization triggered by the adhesin−annexin interaction. The main *S. aureus* invasion process involves a ternary bridge between bacterial FnBP adhesins, fibronectin (Fn), and integrin $\alpha5\beta1$ on human cells.[70,71] Fn-binding by FnBPs disrupts specific intermolecular contacts in the Fn N-terminal domain resulting in structural rearrangements at sites involved in integrin recognition.[72] Then, endocytosis occurs through clathrin-coated pits through rearrangement of the cytoskeleton[73] and F-actin polymerization.[74,75] Aside from this classical pathway, there are other secondary mechanisms of invasion[76] including the ClfA/B-annexin A2 system investigated here. Currently, the molecular details of annexin A2-mediated invasion remain essentially unknown. An important open question is whether binding of ClfA/B elicits molecular changes in annexin A2, activating the internalization process, as for the FnBP-Fn complex. Moreover, as annexin A2 participates in the organization of lipid rafts at sites of actin recruitment,[65] adhesin−annexin raft complexes could trigger a

https://doi.org/10.1021/acs.nanolett.3c01387
Nano Lett. 2023, 23, 5297−5306

raft-dependent biochemical pathway in host cells. It is also worth noting that *E. coli* strains infecting epithelial cells can reorganize the cytoskeleton via annexin A2, which is known to regulate actin dynamics at the bacterium contact.[77,78] Annexin A2 is recruited to the membrane adhesion site by *E. coli*, where F-actin bundling activity is enhanced.[79,80] Annexin A2 also promotes cytoskeletal rearrangements during host cell invasion by *Salmonella*,[30] which is reminiscent of the FnBP-dependent internalization mechanism. We therefore suggest that a similar scenario may occur in which ClfA/B-annexin A2 complexes induce cytoskeletal rearrangement and F-actin polymerization, and the subsequent events leading in *S. aureus* internalization.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.nanolett.3c01387.

Detailed information about methods. Figure S1, adhesion forces between annexin A2-modified tips and *S. aureus* bacteria expressing ClfA or ClfB. Figure S2, localization of ClfA and ClfB adhesins on *S. aureus* bacteria. Figure S3, influence of tip functionalization procedures. Figure S4, adhesion forces of bacteria lacking both ClfA and ClfB. Figure S5, adhesion forces of *S. aureus* $ClfA_{PY}$ bacteria. Figure S6, survival plots obtained for different clamping forces for ClfA and ClfB (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Pietro Speziale − *Department of Molecular Medicine, Biochemistry Unit, University of Pavia, 27100 Pavia, Italy*; Email: pietro.speziale@unipv.it

Giampiero Pietrocola − *Department of Molecular Medicine, Biochemistry Unit, University of Pavia, 27100 Pavia, Italy*; orcid.org/0000-0002-7069-8155; Email: giampiero.pietrocola@unipv.it

Yves F. Dufrêne − *Louvain Institute of Biomolecular Science and Technology, UCLouvain, B-1348 Louvain-la-Neuve, Belgium*; orcid.org/0000-0002-7289-4248; Email: yves.dufrene@uclouvain.be

Authors

Can Wang − *Louvain Institute of Biomolecular Science and Technology, UCLouvain, B-1348 Louvain-la-Neuve, Belgium*

Telmo O. Paiva − *Louvain Institute of Biomolecular Science and Technology, UCLouvain, B-1348 Louvain-la-Neuve, Belgium*

Chiara Motta − *Department of Molecular Medicine, Biochemistry Unit, University of Pavia, 27100 Pavia, Italy*; orcid.org/0000-0001-9276-0936

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.nanolett.3c01387

Author Contributions

C.W., T.O.P., C.M., P.S., G.P., and Y.F.D. designed the experiments, analyzed the data and wrote the article. C.W. and C.M. collected the data.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Work at the Université catholique de Louvain was supported by the National Fund for Scientific Research (FNRS). Funding by the Italian Ministry of University, Project "One Health Basic and Translational Research Actions (INF-Act)", grant # PE13_INFACT_PNRR-U.A. 14.01, CUP F13C22001220007 to G. P. is ackowledged. Y.F.D. is a Research Director at the FNRS. We thank Dr. Joan Geoghegan for providing bacterial strains and Dr. Albertus Viljoen and Dr. M. Mathelié-Guinlet for fruitful discussions.

■ REFERENCES

(1) Méndez-Barbero, N.; San Sebastian-Jaraba, I.; Blázquez-Serra, R.; Martín-Ventura, J. L.; Blanco-Colio, L. M. Annexins and Cardiovascular Diseases: beyond Membrane Trafficking and Repair. *Front. Cell Dev. Biol.* **2022**, *10*, 2093.

(2) Bharadwaj, A.; Kempster, E.; Waisman, D. M. The Annexin A2/S100A10 Complex: the Mutualistic Symbiosis of Two Distinct Proteins. *Biomolecules* **2021**, *11* (12), 1849.

(3) Schloer, S.; Pajonczyk, D.; Rescher, U. Annexins in Translational Research: Hidden Treasures to Be Found. *Int. J. Mol. Sci.* **2018**, *19* (6), 1781.

(4) Bharadwaj, A.; Bydoun, M.; Holloway, R.; Waisman, D. Annexin A2 Heterotetramer: Structure and Function. *Int. J. Mol. Sci.* **2013**, *14* (3), 6259−6305.

(5) Hollås, H.; Ramirez, J.; Nominé, Y.; Kostmann, C.; Toto, A.; Gianni, S.; Travé, G.; Vedeler, A. The Cooperative Folding of Annexin A2 Relies on a Transient Nonnative Intermediate. *Biophys. J.* **2022**, *121* (23), 4492−4504.

(6) Hakobyan, D.; Gerke, V.; Heuer, A. Modeling of Annexin A2-Membrane Interactions by Molecular Dynamics Simulations. *PLoS One* **2017**, *12* (9), e0185440.

(7) Filipenko, N. R.; MacLeod, T. J.; Yoon, C. S.; Waisman, D. M. Annexin A2 Is a Novel RNA-Binding Protein. *J. Biol. Chem.* **2004**, *279* (10), 8723−8731.

(8) Hollås, H.; Aukrust, I.; Grimmer, S.; Strand, E.; Flatmark, T.; Vedeler, A. Annexin A2 Recognises a Specific Region in the 3′-UTR of Its Cognate Messenger RNA. *Biochim. Biophys. Acta-Mol. Cell Res.* **2006**, *1763* (11), 1325−1334.

(9) Kwak, H.; Park, M. W.; Jeong, S. Annexin A2 Binds RNA and Reduces the Frameshifting Efficiency of Infectious Bronchitis Virus. *PLoS One* **2011**, *6* (8), e24067.

(10) Gerke, V.; Weber, K. Identity of P36K Phosphorylated upon Rous Sarcoma Virus Transformation with a Protein Purified from Brush Borders; Calcium-Dependent Binding to Non-Erythroid Spectrin and F-Actin. *EMBO J.* **1984**, *3* (1), 227−233.

(11) Glenney, J. Phospholipid-Dependent Ca^{2+} Binding by the 36-KDa Tyrosine Kinase Substrate (Calpactin) and Its 33-KDa Core. *J. Biol. Chem.* **1986**, *261* (16), 7247−7252.

(12) Hayes, M. J.; Shao, D. M.; Grieve, A.; Levine, T.; Bailly, M.; Moss, S. E. Annexin A2 at the Interface between F-Actin and Membranes Enriched in Phosphatidylinositol 4,5,-Bisphosphate. *Biochim. Biophys. Acta - Mol. Cell Res.* **2009**, *1793* (6), 1086−1095.

(13) Kassam, G.; Manro, A.; Braat, C. E.; Louie, P.; Fitzpatrick, S. L.; Waisman, D. M. Characterization of the Heparin Binding Properties of Annexin II Tetramer. *J. Biol. Chem.* **1997**, *272* (24), 15093−15100.

(14) Zokas, L.; Glenney, J. R. The Calpactin Light Chain Is Tightly Linked to the Cytoskeletal Form of Calpactin I: Studies Using Monoclonal Antibodies to Calpactin Subunits. *J. Cell Biol.* **1987**, *105* (5), 2111−2121.

(15) Taylor, J. R.; Skeate, J. G.; Kast, W. M. Annexin A2 in Virus Infection. *Front. Microbiol.* **2018**, *9*, 2954.

(16) Dallacasagrande, V.; Hajjar, K. A. Annexin A2 in Inflammation and Host Defense. *Cells* **2020**, *9* (6), 1499.

(17) Wright, J. F.; Kurosky, A.; Wasi, S. An Endothelial Cell-Surface Form of Annexin II Binds Human Cytomegalovirus. *Biochem. Biophys. Res. Commun.* **1994**, *198* (3), 983−989.

(18) Derry, M. C.; Sutherland, M. R.; Restall, C. M.; Waisman, D. M.; Pryzdial, E. L. G. Annexin 2-Mediated Enhancement of Cytomegalovirus Infection Opposes Inhibition by Annexin 1 or Annexin 5. *J. Gen. Virol.* **2007**, *88* (1), 19−27.
(19) Raynor, C. M.; Wright, J. F.; Waisman, D. M.; Pryzdial, E. L. G. Annexin II Enhances Cytomegalovirus Binding and Fusion to Phospholipid Membranes. *Biochemistry* **1999**, *38* (16), 5089−5095.
(20) Woodham, A. W.; da Silva, D. M.; Skeate, J. G.; Raff, A. B.; Ambroso, M. R.; Brand, H. E.; Isas, J. M.; Langen, R.; Kast, W. M. The S100A10 Subunit of the Annexin A2 Heterotetramer Facilitates L2-Mediated Human Papillomavirus Infection. *PLoS One* **2012**, *7* (8), e43519.
(21) Taylor, J. R.; Fernandez, D. J.; Thornton, S. M.; Skeate, J. G.; Lühen, K. P.; Da Silva, D. M.; Langen, R.; Kast, W. M. Heterotetrameric Annexin A2/S100A10 (A2t) Is Essential for Oncogenic Human Papillomavirus Trafficking and Capsid Disassembly, and Protects Virions from Lysosomal Degradation. *Sci. Rep.* **2018**, *8* (1), 1−15.
(22) Yang, S. L.; Chou, Y. T.; Wu, C. N.; Ho, M. S. Annexin II Binds to Capsid Protein VP1 of Enterovirus 71 and Enhances Viral Infectivity. *J. Virol.* **2011**, *85* (22), 11809−11820.
(23) Kobayashi, K.; Koike, S. Cellular Receptors for Enterovirus A71. *J. Biomed. Sci.* **2020**, *27* (1), 1−12.
(24) Yen, T. Y.; Shih, W. L.; Huang, Y. C.; Lee, J. Te; Huang, L. M.; Chang, L. Y. Polymorphisms in Enterovirus 71 Receptors Associated with Susceptibility and Clinical Severity. *PLoS One* **2018**, *13* (11), e0206769.
(25) Fang, Y. T.; Lin, C. F.; Liao, P. C.; Kuo, Y. M.; Wang, S.; Yeh, T. M.; Shieh, C. C. K.; Su, I. J.; Lei, H. Y.; Lin, Y. S. Annexin A2 on Lung Epithelial Cell Surface Is Recognized by Severe Acute Respiratory Syndrome-Associated Coronavirus Spike Domain 2 Antibodies. *Mol. Immunol.* **2010**, *47* (5), 1000−1009.
(26) Ma, Y.; Sun, J.; Gu, L.; Bao, H.; Zhao, Y.; Shi, L.; Yao, W.; Tian, G.; Wang, X.; Chen, H. Annexin A2 (ANXA2) Interacts with Nonstructural Protein 1 and Promotes the Replication of Highly Pathogenic H5N1 Avian Influenza Virus. *BMC Microbiol.* **2017**, *17* (1), 1−9.
(27) Kirschnek, S.; Adams, C.; Gulbins, E. Annexin II Is a Novel Receptor for *Pseudomonas aeruginosa*. *Biochem. Biophys. Res. Commun.* **2005**, *327* (3), 900−906.
(28) Munera, D.; Martinez, E.; Varyukhina, S.; Mahajan, A.; Ayala-Sanmartin, J.; Frankel, G. Recruitment and Membrane Interactions of Host Cell Proteins during Attachment of Enteropathogenic and Enterohaemorrhagic *Escherichia coli*. *Biochem. J.* **2012**, *445* (3), 383−392.
(29) Li, X.; Pei, G.; Zhang, L.; Cao, Y.; Wang, J.; Yu, L.; Dianjun, W.; Gao, S.; Zhang, Z. S.; Yao, Z.; Wang, Q. Compounds Targeting YadC of Uropathogenic *Escherichia coli* and Its Host Receptor Annexin A2 Decrease Bacterial Colonization in Bladder. *EBioMedicine* **2019**, *50*, 23−33.
(30) Jolly, C.; Winfree, S.; Hansen, B.; Steele-Mortimer, O. The Annexin A2/P11 Complex Is Required for Efficient Invasion of *Salmonella typhimurium* in Epithelial Cells. *Cell. Microbiol.* **2014**, *16* (1), 64−77.
(31) He, X.; Zhang, W.; Chang, Q.; Su, Z.; Gong, D.; Zhou, Y.; Xiao, J.; Drelich, A.; Liu, Y.; Popov, V.; Zhao, X.; Wakamiya, M.; Gaitas, A.; Lu, F.; Gong, B. A New Role for Host Annexin A2 in Establishing Bacterial Adhesion to Vascular Endothelial Cells: Lines of Evidence from Atomic Force Microscopy and an in Vivo Study. *Lab. Investig.* **2019**, *99* (11), 1650−1660.
(32) Somarajan, S. R.; Al-Asadi, F.; Ramasamy, K.; Pandranki, L.; Baseman, J. B.; Kannan, T. R. Annexin A2Mediates *Mycoplasma pneumoniae* Community-Acquired Respiratory Distress Syndrome Toxin Binding to Eukaryotic Cells. *mBio* **2014**, *5* (4), e01497−14.
(33) Jang, A. Y.; Seo, H. S.; Lin, S.; Chung, G. H.; Kim, H. W.; Lim, S.; Zhao, L.; Park, I. H.; Lim, J. H.; Kim, K. H. Molecular Characterization of Pneumococcal Surface Protein K, a Potential Pneumococcal Vaccine Antigen. *Virulence* **2017**, *8* (6), 875−890.
(34) Hu, Y.; Park, N.; Seo, K. S.; Park, J. Y.; Somarathne, R. P.; Olivier, A. K.; Fitzkee, N. C.; Thornton, J. A. Pneumococcal Surface Adhesion A Protein (PsaA) Interacts with Human Annexin A2 on Airway Epithelial Cells. *Virulence* **2021**, *12* (1), 1841−1854.
(35) Ashraf, S.; Cheng, J.; Zhao, X. Clumping Factor A of *Staphylococcus aureus* Interacts with AnnexinA2 on Mammary Epithelial Cells. *Sci. Rep.* **2017**, *7* (1), 40608.
(36) Ying, Y. T.; Ren, W. J.; Tan, X.; Yang, J.; Liu, R.; Du, A. F. Annexin A2-Mediated Internalization of *Staphylococcus aureus* into Bovine Mammary Epithelial Cells Requires Its Interaction with Clumping Factor B. *Microorganisms* **2021**, *9* (10), 2090.
(37) Peterson, E. A.; Sutherland, M. R.; Nesheim, M. E.; Pryzdial, E. L. G. Thrombin Induces Endothelial Cell-Surface Exposure of the Plasminogen Receptor Annexin 2. *J. Cell Sci.* **2003**, *116* (12), 2399−2408.
(38) Herman, P.; El-Kirat-Chatel, S.; Beaussart, A.; Geoghegan, J. A.; Foster, T. J.; Dufrêne, Y. F. The Binding Force of the Staphylococcal Adhesin SdrG Is Remarkably Strong. *Mol. Microbiol.* **2014**, *93* (2), 356−368.
(39) Herman-Bausier, P.; Dufrêne, Y. F. Force Matters in Hospital-Acquired Infections. *Science* **2018**, *359* (6383), 1464−1465.
(40) Milles, L. F.; Schulten, K.; Gaub, H. E.; Bernardi, R. C. Molecular Mechanism of Extreme Mechanostability in a Pathogen Adhesin. *Science* **2018**, *359* (6383), 1527−1533.
(41) Herman-Bausier, P.; Labate, C.; Towell, A. M.; Derclaye, S.; Geoghegan, J. A.; Dufrêne, Y. F. *Staphylococcus aureus* Clumping Factor A Is a Force-Sensitive Molecular Switch that Activates Bacterial Adhesion. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115* (21), 5564−5569.
(42) Vitry, P.; Valotteau, C.; Feuillie, C.; Bernard, S.; Alsteens, D.; Geoghegan, J. A.; Dufrêne, F. Force-induced Strengthening of the Interaction Between *Staphylococcus aureus* Clumping Factor B and loricrin. *mBio* **2017**, *8* (6), e01748−17.
(43) Merkel, R.; Nassoy, P.; Leung, A.; Ritchie, K.; Evans, E. Energy Landscapes of Receptor-Ligand Bonds Explored with Dynamic Force Spectroscopy. *Nature* **1999**, *397* (6714), 50−53.
(44) Geoghegan, J. A.; Ganesh, V. K.; Smeds, E.; Liang, X.; Höök, M.; Foster, T. J. Molecular Characterization of the Interaction of Staphylococcal Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMM) ClfA and Fbl with Fibrinogen. *J. Biol. Chem.* **2010**, *285* (9), 6208−6216.
(45) McDevitt, D.; Francois, P.; Vaudaux, P.; Foster, T. J. Molecular Characterization of the Clumping Factor (Fibrinogen Receptor) of *Staphylococcus aureus*. *Mol. Microbiol.* **1994**, *11* (2), 237−248.
(46) McDevitt, D.; Francois, P.; Vaudaux, P.; Foster, T. J. Identification of the Ligand-binding Domain of the Surface-located Fibrinogen Receptor (Clumping Factor) of *Staphylococcus aureus*. *Mol. Microbiol.* **1995**, *16* (5), 895−907.
(47) Ganesh, V. K.; Rivera, J. J.; Smeds, E.; Ko, Y. P.; Bowden, M. G.; Wann, E. R.; Gurusiddappa, S.; Fitzgerald, J. R.; Höök, M. A Structural Model of the *Staphylococcus aureus* ClfA-Fibrinogen Interaction Opens New Avenues for the Design of Anti-Staphylococcal Therapeutics. *PLoS Pathog.* **2008**, *4* (11), e1000226.
(48) Mathelié-Guinlet, M.; Viela, F.; Pietrocola, G.; Speziale, P.; Alsteens, D.; Dufrêne, Y. F. Force-Clamp Spectroscopy Identifies a Catch Bond Mechanism in a Gram-Positive Pathogen. *Nat. Commun.* **2020**, *11* (1), 5431.
(49) Melo, M. C. R.; Gomes, D. E. B.; Bernardi, R. C. Molecular Origins of Force-Dependent Protein Complex Stabilization during Bacterial Infections. *J. Am. Chem. Soc.* **2023**, *145* (1), 70−77.
(50) Prystopiuk, V.; Feuillie, C.; Herman-Bausier, P.; Viela, F.; Alsteens, D.; Pietrocola, G.; Speziale, P.; Dufrêne, Y. F. Mechanical Forces Guiding *Staphylococcus aureus* Cellular Invasion. *ACS Nano* **2018**, *12* (4), 3609−3622.
(51) Marshall, B. T.; Long, M.; Piper, J. W.; Yago, T.; McEver, R. P.; Zhu, C. Direct Observation of Catch Bonds Involving Cell-Adhesion Molecules. *Nature* **2003**, *423* (6936), 190−193.
(52) Yakovenko, O.; Sharma, S.; Forero, M.; Tchesnokova, V.; Aprikian, P.; Kidd, B.; Mach, A.; Vogel, V.; Sokurenko, E.; Thomas,

https://doi.org/10.1021/acs.nanolett.3c01387
Nano Lett. 2023, 23, 5297−5306

W. E. FimH Forms Catch Bonds that Are Enhanced by Mechanical Force Due to Allosteric Regulation. *J. Biol. Chem.* **2008**, *283* (17), 11596−11605.
(53) Sokurenko, E. V.; Vogel, V.; Thomas, W. E. Catch-Bond Mechanism of Force-Enhanced Adhesion: Counterintuitive, Elusive, but··· Widespread? *Cell Host Microbe* **2008**, *4* (4), 314−323.
(54) Liu, B.; Chen, W.; Evavold, B. D.; Zhu, C. Accumulation of Dynamic Catch Bonds between TCR and Agonist Peptide-MHC Triggers T Cell Signaling. *Cell* **2014**, *157* (2), 357−368.
(55) Mathelié-Guinlet, M.; Viela, F.; Alsteens, D.; Dufrêne, Y. F. Stress-Induced Catch-Bonds to Enhance Bacterial Adhesion. *Trends Microbiol.* **2021**, *29* (4), 286−288.
(56) Paiva, T. O.; Geoghegan, J. A.; Dufrêne, Y. F. High-Force Catch Bonds between the *Staphylococcus aureus* Surface Protein SdrE and Complement Regulator Factor H Drive Immune Evasion. *Commun. Biol.* **2023**, *6* (1), 302.
(57) Otto, M. *Staphylococcus epidermidis* - The "accidental" Pathogen. *Nat. Rev. Microbiol.* **2009**, *7* (8), 555−567.
(58) Otto, M. Physical Stress and Bacterial Colonization. *FEMS Microbiol. Rev.* **2014**, *38* (6), 1250−1270.
(59) Dufrêne, Y. F.; Persat, A. Mechanomicrobiology: How Bacteria Sense and Respond to Forces. *Nat. Rev. Microbiol.* **2020**, *18* (4), 227−240.
(60) Cheung, G. Y. C.; Bae, J. S.; Otto, M. Pathogenicity and Virulence of *Staphylococcus aureus*. *Virulence* **2021**, *12* (1), 547−569.
(61) Milles, L. F.; Unterauer, E. M.; Nicolaus, T.; Gaub, H. E. Calcium Stabilizes the Strongest Protein Fold. *Nat. Commun.* **2018**, *9* (1), 4764.
(62) Milles, L. F.; Gaub, H. E. Extreme Mechanical Stability in Protein Complexes. *Curr. Opin. Struct. Biol.* **2020**, *60*, 124−130.
(63) Huang, W.; Le, S.; Sun, Y.; Lin, D. J.; Yao, M.; Shi, Y.; Yan, J. Mechanical Stabilization of a Bacterial Adhesion Complex. *J. Am. Chem. Soc.* **2022**, *144* (37), 16808−16818.
(64) Foster, T. J.; Geoghegan, J. A.; Ganesh, V. K.; Höök, M. Adhesion, Invasion and Evasion: the Many Functions of the Surface Proteins of *Staphylococcus aureus*. *Nat. Rev. Microbiol.* **2014**, *12* (1), 49−62.
(65) Gerke, V.; Creutz, C. E.; Moss, S. E. Annexins: Linking Ca^{2+} Signalling to Membrane Dynamics. *Nat. Rev. Mol. Cell Biol.* **2005**, *6* (6), 449−461.
(66) Concha, A. N. O.; Head, J. F.; Kaetzel, M. A.; Dedman, J. R.; Seaton, B. A. Rat Annexin V Crystal Structure: Ca^{2+} Induced Conformational Changes. *Science* **1993**, *261* (5126), 1321−1324.
(67) Wice, B. M.; Gordon, J. I. A Strategy for Isolation of CDNAs Encoding Proteins Affecting Human Intestinal Epithelial Cell Growth and Differentiation: Characterization of a Novel Gut-Specific N-Myristoylated Annexin. *J. Cell Biol.* **1992**, *116* (2), 405−422.
(68) Valapala, M.; Vishwanatha, J. K. Lipid Raft Endocytosis and Exosomal Transport Facilitate Extracellular Trafficking of Annexin A2. *J. Biol. Chem.* **2011**, *286* (35), 30911−30925.
(69) Matos, A. L. L.; Kudruk, S.; Moratz, J.; Heflik, M.; Grill, D.; Ravoo, B. J.; Gerke, V. Membrane Binding Promotes Annexin A2 Oligomerization. *Cells* **2020**, *9* (5), 1169.
(70) Joh, D.; Wann, E. R.; Kreikemeyer, B.; Speziale, P.; Höök, M. Role of Fibronectin-Binding MSCRAMMs in Bacterial Adherence and Entry into Mammalian Cells. *Matrix Biol.* **1999**, *18* (3), 211−223.
(71) Sinha, B.; François, P. P.; Nüße, O.; Foti, M.; Hartford, O. M.; Vaudaux, P.; Foster, T. J.; Lew, D. P.; Herrmann, M.; Krause, K. H. Fibronectin-Binding Protein Acts as *Staphylococcus aureus* Invasin *via* Fibronectin Bridging to Integrin $\alpha5\beta1$. *Cell. Microbiol.* **1999**, *1* (2), 101−117.
(72) Liang, X.; Garcia, B. L.; Visai, L.; Prabhakaran, S.; Meenan, N. A. G.; Potts, J. R.; Humphries, M. J.; Höök, M. Allosteric Regulation of Fibronectin/$\alpha5$ $\beta1$ Interaction by Fibronectin-Binding MSCRAMMs. *PLoS One* **2016**, *11* (7), e0159118.
(73) Sinha, B.; Fraunholz, M. *Staphylococcus aureus* Host Cell Invasion and Post-Invasion Events. *Int. J. Med. Microbiol.* **2010**, *300* (2−3), 170−175.
(74) Ellington, J. K.; Reilly, S. S.; Ramp, W. K.; Smeltzer, M. S.; Kellam, J. F.; Hudson, M. C. Mechanisms of *Staphylococcus aureus* Invasion of Cultured Osteoblasts. *Microb. Pathog.* **1999**, *26* (6), 317−323.
(75) Jevon, M.; Guo, C.; Ma, B.; Mordan, N.; Nair, S. P.; Harris, M.; Henderson, B.; Bentley, G.; Meghji, S. Mechanisms of Internalization of *Staphylococcus aureus* by Cultured Human Osteoblasts. *Infect. Immun.* **1999**, *67* (5), 2677−2681.
(76) Josse, J.; Laurent, F.; Diot, A. Staphylococcal Adhesion and Host Cell Invasion: Fibronectin-Binding and Other Mechanisms. *Front. Microbiol.* **2017**, *8*, 2433.
(77) Hayes, M. J.; Rescher, U.; Gerke, V.; Moss, S. E. Annexin-actin Interactions. *Traffic* **2004**, *5* (8), 571−576.
(78) Hayes, M. J.; Shao, D.; Bailly, M.; Moss, S. E. Regulation of Actin Dynamics by Annexin 2. *EMBO J.* **2006**, *25* (9), 1816−1826.
(79) Miyahara, A.; Nakanishi, N.; Ooka, T.; Hayashi, T.; Sugimoto, N.; Tobe, T. Enterohemorrhagic *Escherichia Coli* Effector EspL2 Induces Actin Microfilament Aggregation through Annexin 2 Activation. *Cell. Microbiol.* **2009**, *11* (2), 337−350.
(80) Tobe, T. Cytoskeleton-Modulating Effectors of Enteropathogenic and Enterohemorrhagic *Escherichia Coli*: Role of EspL2 in Adherence and an Alternative Pathway for Modulating Cytoskeleton through Annexin A2 Function. *FEBS J.* **2010**, *277* (11), 2403−2408.

https://doi.org/10.1021/acs.nanolett.3c01387
Nano Lett. 2023, 23, 5297−5306