



HAL
open science

Recent Advances and Emerging Challenges in the Molecular Modeling of Mechanobiological Processes

Guillaume Stirnemann

► **To cite this version:**

Guillaume Stirnemann. Recent Advances and Emerging Challenges in the Molecular Modeling of Mechanobiological Processes. *Journal of Physical Chemistry B*, 2022, 126 (7), pp.1365-1374. 10.1021/acs.jpcc.1c10715 . hal-03826629

HAL Id: hal-03826629

<https://hal.science/hal-03826629v1>

Submitted on 8 Nov 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Recent advances and emerging challenges in the molecular modeling of mechanobiological processes

Guillaume Stirnemann*

*CNRS Laboratoire de Biochimie Théorique, Institut de Biologie Physico-Chimique, PSL
University, Université de Paris, 13 rue Pierre et Marie Curie, 75005, Paris, France*

E-mail: stirnemann@ibpc.fr

Abstract

Many biological processes result from the effect of mechanical forces on macromolecular structures and on their interactions. In particular, the cell shape, motion and differentiation, directly depend on mechanical stimuli from the extra-cellular matrix or from neighbouring cells. The development of experimental techniques that can measure and characterize the tiny forces acting at the cellular scale and down to the single-molecule, biomolecular level, has enabled access to unprecedented details about the involved mechanisms. However, because the experimental observables often do not provide a direct atomistic picture of the corresponding phenomena, particle-based simulations performed at various scales are instrumental in complementing these experiments and in providing a molecular interpretation. Here, we will review the recent key achievements in the field, and we will highlight and discuss the many technical challenges these simulations are facing, as well as suggest future directions for improvement.

Introduction

At the microscopic level, the detailed knowledge of interatomic forces, such as chemical bonds, electrostatic interactions, or van der Waals forces, is often both irrelevant (as the central concept of statistical thermodynamics is to identify the thermodynamic properties stochastically emerging from the microscopic behavior of molecules) and not easily measured in the experiments. Instead, the concept of *mechanical* forces acting on biomolecular objects however becomes of direct relevance (and measurable) when considering a variety of interactions between macromolecules, cells, and their environments. The final form of a cell and of a multicellular organism depends on mechanotransduction, which is the cascade of mechanisms converting mechanical stimuli into biochemical signals that regulate the cell functions, including its shape, its motion or its differentiation.¹ The generation of macroscopic forces by muscles depends on the extension and contraction of the biomolecules forming its con-

tractile units.^{2,3} Other key biological mechanisms, including blood coagulation⁴ or urinary infections,⁵ are regulated by the effect of shear flow on large biomolecular assemblies.

Owing to the development of elaborate experimental tools⁶⁻⁹ to apply and measure forces in a range that is relevant at the biomolecular and cellular level (typically, from hundreds of nN down to the pN regime), spectacular progress has been made in the understanding of the molecular processes of mechanobiology, and of how forces are generated by (and in turn affect) the macromolecular players of the cell.¹ In particular, the emergence of single-molecule force spectroscopy techniques in the last 3 decades has allowed the manipulation of these biomolecular units one by one.⁶ This evolution coincided with the development of approaches based on computer simulations at a molecular level that have complemented and helped interpret the experimental results and unravel the associated molecular mechanisms.^{10,11}

Some 25 years after these first steered molecular dynamics simulations,^{12,13} we will discuss some key recent advances and some emerging challenges in the molecular modeling of mechanical processes in biology. This article is not meant to be an extensive review of the discipline; it will instead focus on its latest developments and on the current open questions. It will be restricted to biophysical phenomena, i.e., it will not discuss the direct effect of forces on chemical reactions, including some that could occur in the cell, and the interested reader is referred to recent reviews.^{14,15} We chose to cover a broad range of simulations, from fully atomistic up to more coarse-grained mesoscopic approaches, because experimental studies span several orders of magnitude in time- and lengthscale that require adapted computational tools. We propose to focus on five different aspects that are at the forefront of the field today, with a special focus on proteins or proteins assemblies, leaving aside studies dealing more specifically with nucleic acids or membranes. In each case, we will discuss the general *in vivo* and *in vitro* experimental context as well as the appropriate simulation strategies and the recent milestones. In particular, the choice of a simulation approach depends on the nature of the force (e.g. a direct mechanical force between two biomolecules, a shear force exerted by a fluid in motion, etc.), its directionality and way of application (which is easily

controlled in single-molecule force-spectroscopy experiments but less so *in vivo*), its amplitude, the size of the involved biomolecules, and the general biophysical context (isolated proteins versus complex biomolecular complexes).

Protein conformational changes upon application of high directional forces

During the 90s, it became possible to measure forces and displacements generated by biomolecular objects at the single molecule level using force spectroscopy techniques such as the atomic force microscope (AFM), optical tweezers (OT) or magnetic tweezers (MT).⁶ Although some of these techniques also enable more specific modes of force application (such as twisting or force-ramps), most of the time, they are used to stretch molecules attached at their two extremities at constant pulling velocity or at constant force. These techniques have revolutionized the study of biomolecules by allowing to manipulate these objects one by one, highlighting the stochastic nature of events occurring at these scales that could not be evidenced by bulk techniques. While force is of direct biological relevance for a variety of these molecules, such as the constituents of muscle fibers that are subject to directional mechanical force, the *in vivo* implications for many other systems that were studied by these techniques is much less obvious. In these cases, force is used as a way to perturb and thus to probe the biomolecule properties, in particular, its conformational plasticity ranging from small deformation up to complete unfolding.

At the same time, the increase in computational power together with improved models and algorithms has made the molecular dynamics (MD) simulations of biomolecules more and more routinely accessible. At the end of the 90s, a particular class of non-equilibrium MD simulations emerged in order to apply external force on (bio)molecules, mimicking single-molecule force experiments.^{12,13} In a normal MD simulation, atoms typically obey to Newtonian dynamics under controlled thermodynamic conditions, such as total volume or temper-

ature. In steered MD simulations, additional biases are added to the equations of motions: some portions of the proteins may be immobilized (mimicking attachment to a surface) while others can be pulled at constant force, or subject to a harmonic potential moved at constant velocity. The only difference is that there is usually neither pulling nor immobilizing devices in the simulations, while experiments require beads, cantilever tips, piezoelectric crystals, as well as molecular linkers, to attach and to manipulate single biomolecules. Some progress has also been made in the recent years regarding the understanding of the experimental setup effect on the measured kinetics (in particular, that of the flexible linkers¹⁶ and of the tethering object^{17,18}), and how this should be taken into account for the comparison with MD simulation data.

When trying to compare experimental and molecular dynamics simulation results, questions can be raised about the accuracy of the atomistic forcefields, and some recent effort has been devoted to the use of polarizable models.¹⁹ However, non-polarizable biomolecular forcefields have considerably improved, and it is generally accepted that they offer a reliable and robust description of molecular interactions. Even provided with good molecular models, the key fundamental issue in the field of SMD, which also affects MD simulations in general, is that solving the equations of motion numerically requires very small timestepping, strongly limiting the accessible simulation time. Although computational power and strategies (such as the routine use of GPUs today) as well as the deployment of important algorithmic developments in widely distributed codes (e.g., allowing for longer timesteps) lead to a constant improvement of the MD engines efficiency, routine simulations of isolated proteins are typically limited to the microsecond timescale. But the timescale of the experiments is much longer, typically on the order of the second. A direct consequence is that for example, the force under which a given protein unfolds on a second timescale has barely any effect on a microsecond of simulation time (Figure 1). Therefore, simulations typically use much larger forces or much faster pulling velocities as compared to the experiments in order to observe the same events on a shorter time window. Such a strategy

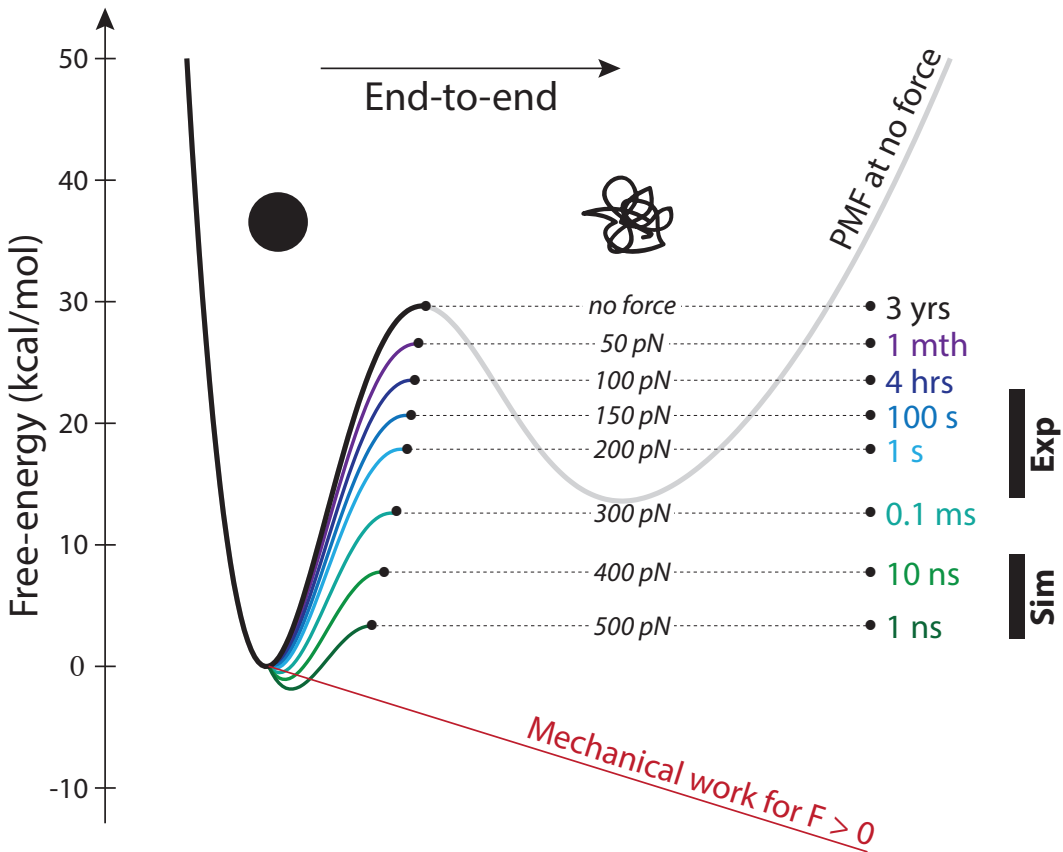


Figure 1: Effect of force on the free-energy landscape of protein folding along the the end-to-end distance. A model, asymmetric double-well potential has been defined as a linear combination of two harmonic potentials describing the unfolded and folded states of a protein, respectively populated and depopulated using position-dependent switching functions. The parameters were tuned such that the free-energy barrier (30 kcal/mol) and the distance to the transition state (0.44 nm) mimic the typical unfolding of a mechanically stable protein. In the absence of force, the barrier cannot be crossed on experimental timescales (gray). Application of a mechanical force results in an additional mechanical work (red line) that lowers the effective free-energy barrier (colored lines). The unfolding times were estimated by applying transition state theory (neglecting diffusion effects) and using the calculated barrier at each force (because of the specific shape of the employed PMF, the distance to the transition state slightly decreases with force). In this example, experimental timescales that are typically on the order of ms-s will observe unfolding for forces close to 150–200 pN. However, the limited timescale of the simulations, typically in the ns- μ s range, implies that unfolding can only be observed at much higher forces, typically 400–500 pN in this example.

raises an important question: to what extent the mechanisms responsible for the experimental signals and measurements at a given force/pulling velocity are identical to those of the simulations performed under stronger perturbations? Thanks to the recent development

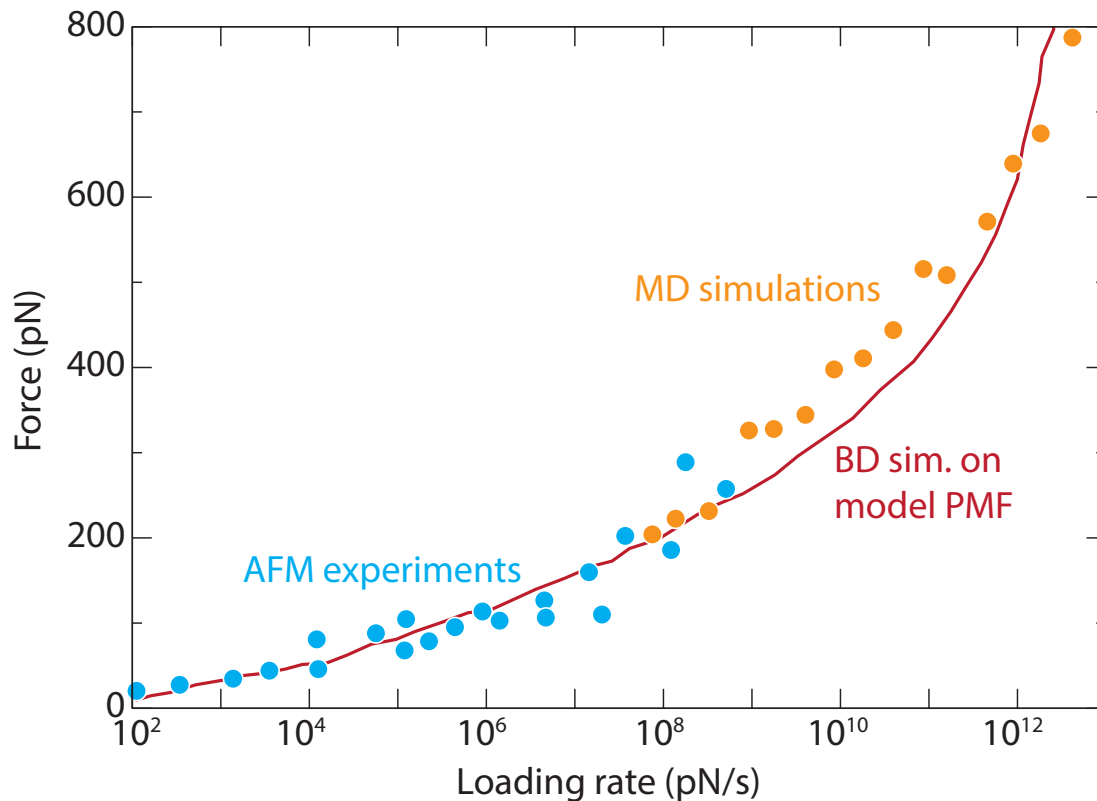


Figure 2: Average rupture force for the biotin–streptavidin dissociation as a function of the loading rate, measured by AFM experiments (blue) and MD simulations (orange) (data taken from ref.²⁰). The red line indicates the results of Brownian dynamics (BD) simulations performed on a model potential that can account for both experimental and simulation results. Quite remarkably, the MD simulations performed at their slowest loading rates agree with experiments performed at their highest loading rates.

of high-speed force-spectroscopy, and thanks to the evolution of computational resources, recent joint experimental/simulation studies of ligand-protein unbinding²⁰ and protein unfolding²¹ have closed the gap of timescales between both approaches (Figure 2). However, while experimental and simulation data that together span orders of magnitude of pulling velocities are well fitted by elaborate models of a single barrier crossing upon force,^{22,23} the biotin-streptavidin unbinding pathways are sensitive to the pulling velocity.²⁰ The effect of the pulling velocity was also recently evidenced on simulations of the mechanical unfolding of polyproteins, where high loading rates lead to biases in the unfolding sequence of the protein repeats.²⁴

When force is applied on a protein that is already unfolded, a direct comparison between all-atom MD simulations and experimental data is possible at the same forces as far as they lie above a few tens of piconewtons, i.e., in a regime where the extended protein behaves as a worm-like chain upon force with no possibility of formation of partially folded intermediates. By recognizing that forces in the 30–500 pN range mainly affect the backbone dihedral degrees of freedom,²⁵ approaches based on SMD simulations and on potentials of mean force tilted upon force could explain the peculiar force-dependence of substrate recognition by a protein chaperone²⁶ or the cleavage of a polypeptide substrate by a protease.²⁷

Another possibility in order to observe experimentally-measured events on the simulation accessible timescale is to employ enhanced sampling strategies specifically adapted for simulations under constant force or pulling velocity, such as boxed molecular dynamics,²⁸ accelerated steered molecular dynamics,²⁹ or infinite switch simulated tempering in force³⁰ (note that techniques that specifically target an indirect estimation of kinetics based on estimations of potentials of mean force will be discussed later). While such strategies appear promising in closing the gap between simulations and experiments, they suffer from some limitations, such as limited sampling along coordinates that are perpendicular to the reaction coordinate (that could relax on timescales slower than that of the simulations) and often the loss of the time-course of a trajectory — preventing the direct extraction of any kinetic quantities and of the order of events in the unfolding/unbinding pathways.

While the first SMD results were based, at best, on a handful of trajectories, SMD trajectories can now be routinely performed multiple times under given conditions.^{24,31–39} Just like experimental data is obtained based on dozens if not hundreds of pulling traces, the stochastic nature of the conformational changes occurring in biomolecular systems at the single-molecule level requires the observation of multiple events in order to draw an average, general picture on the system behavior at a given force/pulling velocity. Interestingly, MD simulations can also be used to compare the effect of the pulling direction. In many in vivo situations as well as in single-molecule force-spectroscopy experiments, one end of the

protein is attached to a large interface or to an immobile substrate, while the other is subject to mechanical stimuli. In most single-molecule experiments, the non specific binding of the protein to the immobile surface and to the mechanical tether implies that there is a mixture of indistinguishable events corresponding to traces where either the N-terminal or the C-terminal was pulled, the other being fixed. In the simulations, this is straightforwardly controlled. In most cases, the pulling direction is not expected to make any difference on the unfolding mechanism, but it has been shown to lead to distinct unfolding pathways for particular systems, such as metalloproteins.³¹

The low force regime

The application of directional mechanical force has enabled the study of protein folding/unfolding at the single-molecule level. However, a key issue is that these techniques impose and probe these processes along one particular reaction coordinate, the end-to-end distance. While early work supported the idea that high-force unfolding data could be extrapolated at zero force and that mechanical and chemical/thermal unfolding were proceeding along the same pathways,⁴⁰ a number of all-atom and coarse-grained MD studies have shown that the folding/unfolding pathways⁴¹⁻⁴³ as well as the unfolded state ensembles are markedly different.⁴⁴ It is therefore not surprising that mechanical and thermal resistance of protein mutants are not necessarily correlated.^{32,33}

Even if the low-force (1-10 pN) regime could readily be accessed using optical tweezers, allowing the observation of multiple folding/unfolding events of the same biomolecule,⁴⁵ the democratisation of magnetic tweezers together with covalent attachment between the beads and the biomolecular objects,^{46,47} have allowed to routinely access very low forces and to manipulate the same single molecule for several hours. At these forces, the reaction pathways might differ from that at high forces, and might even approach the pathways in the absence of force. The usual projection of the conformational free-energy landscape along the end-

to-end distance, and the corresponding theoretical framework,^{22,23} could be complicated by the importance of folding pathways occurring along collective variables not necessarily correlated with the end-to-end distance, and that become relevant at low forces^{14,41} (Figure 3). Recent experimental evidence was recently provided for several protein systems whose unfolding kinetics varied non-monotonously with force,^{48,49} which possibly results from different pathways at low and high forces.

Tackling the low-force regime with MD simulations appears to be more problematic as compared to higher force SMD. Because of slower kinetics, longer simulation timescales are required to observe and to record multiple unfolding events. At low forces, proteins can also refold, which represents an ever greater challenge for molecular dynamics approaches. Approaches based on coarse grained models in implicit solvent are of particular interest in that case, enabling the exploration of the conformational space faster, either by propagating SMD trajectories, or by determining the free-energy landscape under force using enhanced sampling techniques.^{41-43,50-52} Some other work have attempted to determine a multidimensional potential of mean force at zero force based on all-atom MD in explicit solvent,⁵³ but these are currently limited to small and/or model proteins as the phase space sampling along all relevant folding/unfolding coordinates is not easily achieved. Moreover, even multidimensional PMFs require to make some assumptions and choices about the folding coordinates, which become highly not trivial and not easily predicted for typical size proteins. These studies provide overwhelming evidence that the unfolding pathways at low and high forces are in many cases different (Figure 3), with a variety of unfolding pathways and intermediates that cannot be observed under high forces.^{41-43,51,52}

An interesting complement to the approaches discussed above can be provided by Brownian or Langevin dynamics simulations. These simulations can reproduce experimental-like traces of extension versus time. They are either performed on the (typically projected in 1d) free-energy landscapes determined using the approaches detailed above,^{50,53} or, on model PMFs that can help interpret experimental data on polyproteins.^{46,54}

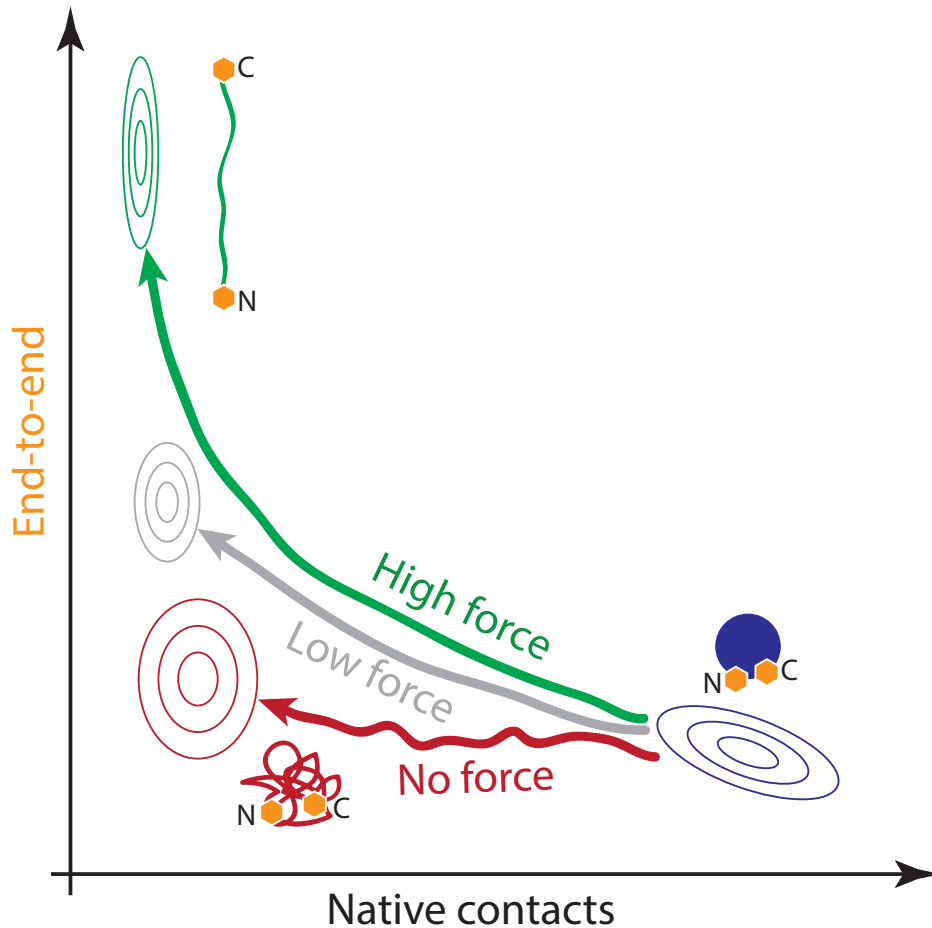


Figure 3: Effect of force on the unfolding pathway as seen from the perspective of a multi-dimensional PMF. The end-to-end (orange) distance is usually not a very relevant reaction coordinate for unfolding in the absence of force (red). Metrics such as the number of native contacts are typically more adapted. In particular, the unfolded "molten-globule" (red noodle) usually has end-to-end distances not very different from that of the native state (blue sphere), but it has barely any of the native contacts found in the folded state. When a high force is applied, unfolding typically proceeds through a very different pathway (green), with a considerable extension of the end-to-end distance and the progressive loss of native contacts, and a final state that is extended (green noodle). When force is lowered (gray), the unfolding pathway becomes closer to the one in the absence of force. Note that this is of course a very schematic picture and that actual pathways and reaction coordinates in the absence of force might be much more complicated.

So far, we have mostly discussed cases where mechanical force is applied along one defined and controlled direction, which is directly achieved in single molecule experiments and in simulations. But in many situations occurring *in vivo* (for example, in mechanotransduc-

tion), defining the exact direction of the force is not straightforward, and it can vary along time. Other biological processes actually depend on the response to fluid motion, both at the cellular and at the biomolecular level. In particular, shear flow can trigger important protein conformational changes that could eventually lead to some counterintuitive behaviors. We now discuss some key progress and open challenges for molecular simulations of, successively, shear flows, catch-bond phenomena, and mechanotransduction.

Shear-induced conformational changes

Hydrodynamic effects play a significant role both at the scale of the cell and consequently, at that of the organism.⁵⁵ Important examples include the regulation of blood pressure by endothelial cells in blood vessels, the sequestration of leucocytes on the vessel walls during the immune response, or the formation of hemostatic plug after an injury. In a non-physiological context, just like directional mechanical force has been used as an experimental probe of biomolecular processes, shear flow in microfluidic devices has been employed e.g. on actin filaments to understand how mechanical stress affects actin assembly.⁵⁶

All these mesoscale manifestations of shear flow are actually a consequence of the flow effect on some of the cell biomolecular constituents, in particular on some key proteins involved in these regulation processes. The understanding of the underlying molecular mechanisms has prompted a number of experimental studies with sometimes contradictory results,^{57,58} and accordingly, to molecular simulations in order to help interpret the results.⁵⁹

However, the effect of solvent shear flow is not necessarily identical to that of a directional mechanical force (Figure 4). Modelling shear flow using all-atom approaches in explicit solvent face some limitations. First, imposing a fluid flow requires to rescale velocities of solvent molecules,⁶⁰ or to mimic experimental setups by using moving interfaces.⁶¹ Second, the sampling problem and the limited timescale of these simulations are similar to that observed when running SMDs at experimental pulling forces. For these reasons, coarse-grained

biomolecular representations in implicit solvent offer a more direct implementation of hydrodynamic effects, and access to longer simulation timescales.^{59,62,63} Significant progress has been made in the recent years, such as the use of a Lattice-Boltzmann scheme for solvent-solute hydrodynamic interactions, and improved coarse-grained models.^{33,64,65} However, important challenges remain, because even long simulation timescales do not allow to observe significant conformational changes at experimental shear flows.^{33,61} Employing enhanced sampling techniques, that are usually deployed in a rigorous thermodynamic framework, is not trivial when using these non-equilibrium techniques, but some interesting attempts can be noticed.⁶⁶

The use of directional forces in the simulations or in the experiments is a tempting and more-easily implemented approach to mimic the effect of shear flow on protein conformations. However, these are very different modes of force applications (Figure 4), as evidenced in a number of comparative studies. Brownian dynamics simulations suggested that the force acting on a surface-grafted protein under shear flow was very negligible at low shear flow but increased sharply as a function of the applied shear flow beyond a threshold value.⁶³ As recently shown on a model protein system, the unfolding pathways under high shear rates were actually closer to thermal unfolding pathways than they were to those under a directional mechanical force.³³ As protein conformations evolve under shear, the exposure of protein regions to the solvent changes, and the propagation of the mechanical constraints along the biomolecular structure also varies.⁶⁷ Recent simulations have suggested that the force felt by the free end of protein subject to a constant shear flow significantly varies along time.⁶⁵

A spectacular manifestation of shear-induced effects on protein conformations and subsequently interactions with other biomolecules is provided by the von Willebrand factor (vWf), a giant multimeric protein involved in hemostasis, through a variety of complementary complex mechanisms.^{4,68} In the absence of shear, the protein adopts a compact, multiglobular state. After a blood vessel cut, the increase in shear flow triggers a cascade of conforma-

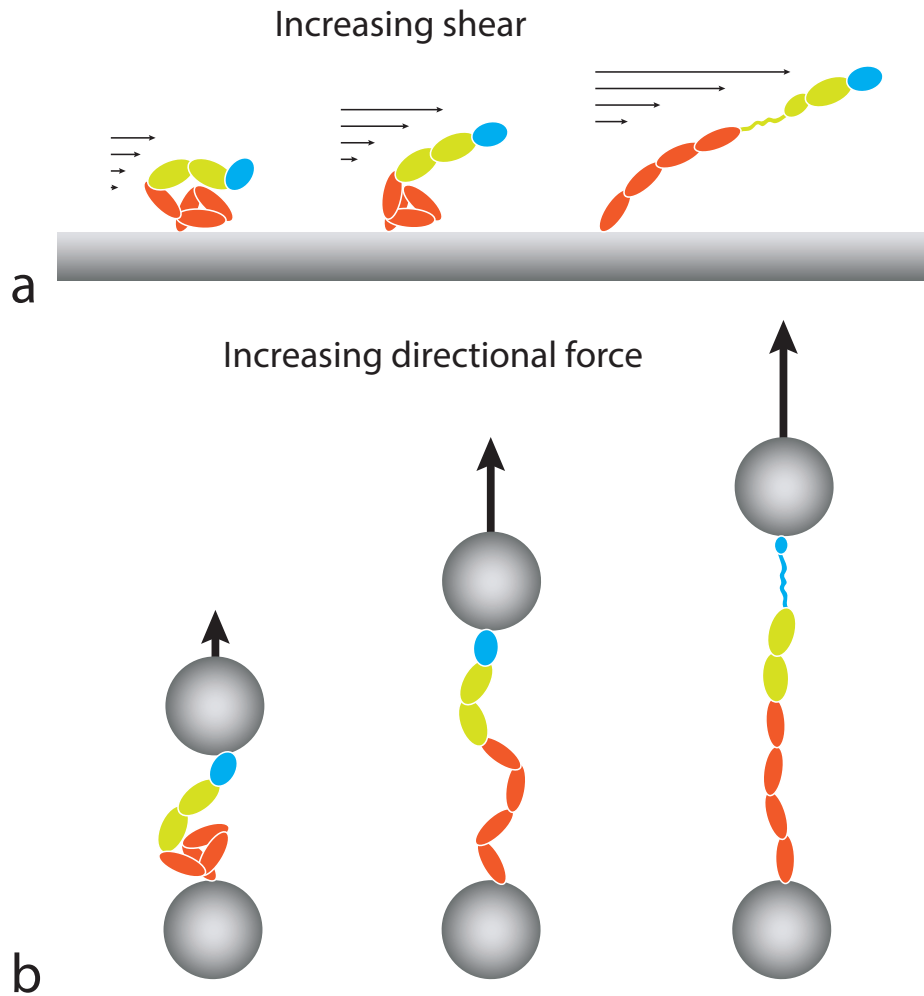


Figure 4: Schematic effect of (a) shear flow and (b) mechanical force of increasing strength on a multidomain protein. The structures obtained with these two modes of force application are not easily comparable, and the mechanical constraints acting on the protein parts are very different. In particular, the mechanical stability of the individual domains need not be the same depending on the force conditions. Here, a green domain unfolds first upon high shear because it may sense more tension, whereas the blue domain is the least mechanical stable in the presence of a directional mechanical force.

tional changes, including elongation⁶⁹ and domain separation⁶³ that lead to the binding of the glycoprotein $Ib\alpha$ subunit that are at the surface of platelets to the A1 domains of the vWf, which eventually results in a hemostatic plug.⁷⁰ Shear flow also acts as a regulator of platelets accumulation by modulating the length of the exposed portion of the vWf, that could lead to thrombosis. Indeed, one of its domains (A2) can unfold upon force, expos-

ing a specific site that is cleaved by a protease, therefore reducing the length of the total protein.⁷¹ Additionally, experimental studies have revealed that the interaction between the A1 domains and the platelet glycoproteins is enhanced by force, which is the signature of a catch-bond system that will be discussed in more details below. While earlier simulations focused on the mechanism of the A1/glycoprotein interaction upon force,^{60,72,73} more recently, larger scale all-atom MD simulations⁷⁴ as well as coarse-grained approaches^{63,65,67,75} have shed light on the molecular mechanisms of the shear effect on domain separation and unfolding upon shear. These questions are not limited to the specific case of the vWf: the peculiar behavior of fibrin (another protein involved in hemostasis) under mechanical load has also recently been investigated using MD simulations.⁷⁶

Catch-bonds

The counter-intuitive enhanced adhesion upon shear flow evidenced for the vWf factor has been evidenced in a growing number of other contexts, such as mechanotransduction and bacterial adhesion.⁷⁷ A particularly well-studied example is the bacterial adhesin FimH that is located at the tip of *E. coli* pili (Figure 5). FimH allows bacteria to anchor to human urinary tract epithelia, potentially causing infections. The catch-bond mechanism allows the bacteria to avoid elimination through miction while maintaining their proliferation capabilities in a quiet environment. The combination of experiments under flow, single-molecule force spectroscopy and crystallographic structures of FimH bound to various ligands have yielded valuable insights on this counter-intuitive catch-bond phenomenon.^{5,78,79} FimH is composed of two domains, a distal lectin domain that contains the binding site, and a proximal pilin domain that is attached to the rest of the pilus rod (Figure 5). The catch-bond behavior is attributed to an intramolecular allosteric mechanism. When the two domains interact in a so-called associated state (A), the binding affinity is low; conversely, upon separation of the domains upon force (separated state S), the affinity is increased (Figure 5). However,

static crystallographic structures provide little clue on the molecular mechanism of the A to S transition, which involves significant local conformational rearrangements not only on the pilin-lectin interfacial region, but also in lectin regions connecting this interface and the binding site. Moreover, the binding site structure of the A and S states are almost superimposable in the X-ray structures,⁷⁹ which does not offer a direct structural explanation to the measured differences in affinity.

MD simulations have focused on the comparison between the different states of the FimH lectin domain. In particular, the plasticity of the binding site was observed to be different in the two states.^{79,80} Long microsecond simulations⁸¹ investigated some key steps of the A to S conformational transition, but the full interconversion between the two states was never achieved on this timescale. Other studies focused on the effect of directional force on the lectin-pilin domains separation,⁸² as well as on the mechanical stability of FimH and of the other pili domains.⁸³ However, important challenges remain for molecular simulations. For example, the free-energy landscape, kinetics, and molecular mechanism of the A to S transition remain unclear. Because of a large free-energy barrier, it can probably only be achieved with enhanced sampling techniques, but the reaction coordinates are not well-characterized. Moreover, in addition to its role as an allosteric trigger, force could also have an impact on the protein microstates in each the A or S states, which in turn could also regulate the affinity. Some of our current efforts on the topic are devoted to these specific questions.

Mechanosensing and mechanotransduction

As more and more emerging technologies allow to measure and to apply forces at the piconewton scale,^{7-9,84} experimental studies are providing many exciting results regarding the mechanisms through which cells can sense forces from their environment^{1,85} and how it triggers a chain of events that affect gene expression, the cell behavior and function, and

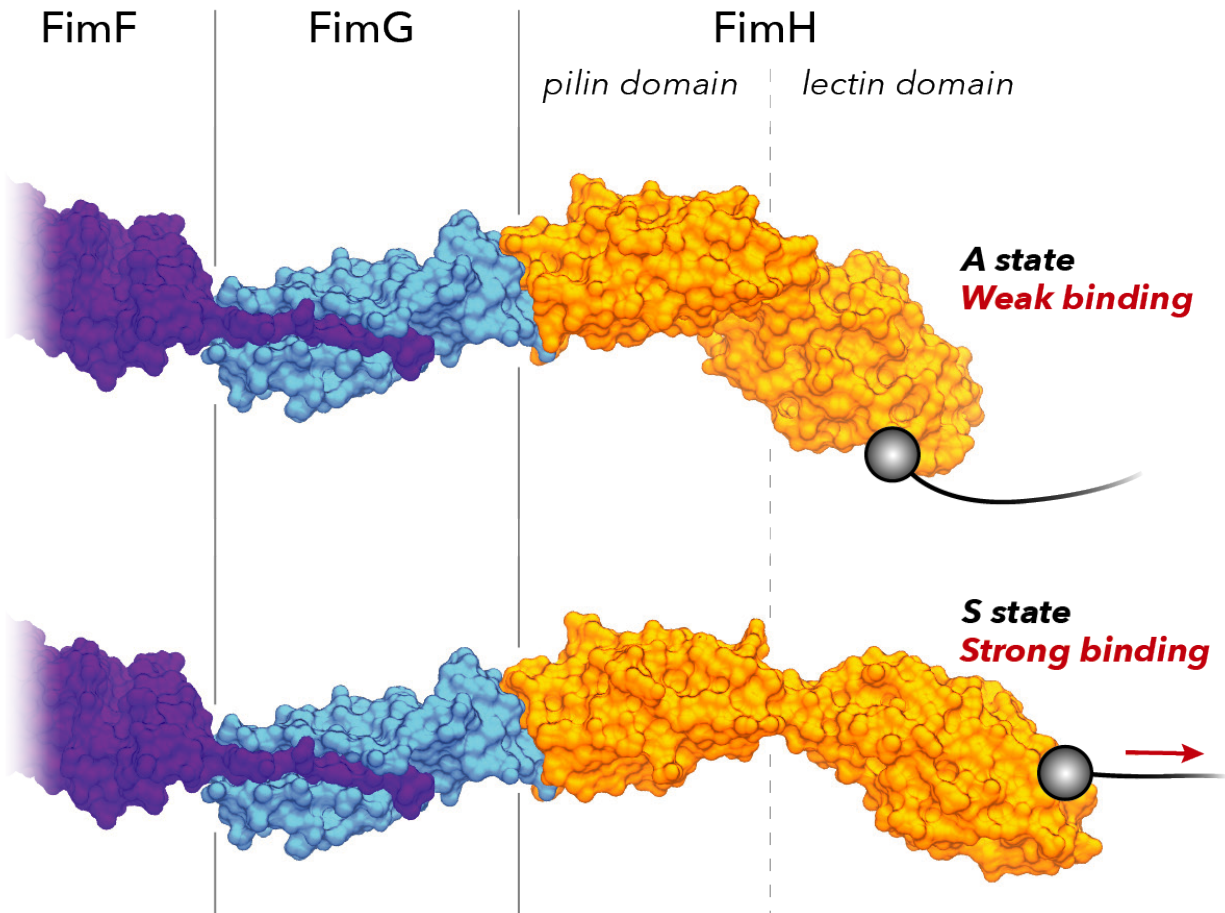


Figure 5: FimH catch-bond mechanism. FimH is located at the end of *E. coli* pilus that is composed of many other Fim domains (left side). FimH (orange) is composed of two domains, the pilin domain that is linked to the rest of the pilus (blue and purple), and the lectin domain, bound to a mannosylated glycan at the surface of an epithelium cell (brown, right). Upon application of shear flow, the assembly switches from a weakly bound state (top), where the two FimH domains are in an associated state, to a strongly bound state (bottom), where the two domains are separated.

eventually the development of the full organism.⁸⁶ It is impossible to review here the large variety of questions and systems in these fields, and only a few examples will be mentioned. These phenomena are highly complex, as they involve many different proteins organized into complicated structures, from the integrins involved in focal adhesion that interact with the extracellular matrix; cadherins involved in cell-cell junctions; sensing proteins such as talin and vinculin; actin and myosin motors that eventually control the shape of the cell; nuclear signalling factors such as YAP or LIM, to name a few examples of some of the key players of

mechanotransduction.¹ Mechanosensing assemblies are involved in other biological processes, for example, in the positioning of the nucleus in the cell,^{87,88} or in the perception of sound and motion.⁸⁹ Finally, some interactions between mechanotransduction's key biomolecular players exhibit a directional catch-bond behavior. When mechanical force is applied to vinculin in complex to actin, resistance is much stronger in one of the actin filament direction and the complex lifetime increases with force.⁹⁰

For molecular simulations, these systems present many of the challenges exemplified in the previous sections: large system sizes, long timescales, varying and not well-defined modes of force application, and typically, of small piconewton-scale amplitude. All-atom MD simulations have been used in some cases, but they employed forces that were much larger than the experimental loads.^{34,38,91-94} Considering the involved time- and length-scales, coarse graining and multiscale approaches are an appealing alternative to fully atomistic simulations.⁹⁵ Recent examples include, but are not limited to, the study of the mechanical factors that govern the positioning of actin-binding proteins on actin filaments,⁹⁶ the morphology of integrin assemblies in cell adhesion,⁹⁷ the dissociation mechanism of cell-adhesion complexes under force,⁹⁸ or the mechanical properties of microtubules.⁹⁹ As suggested in another recent perspective,¹⁰⁰ the combination of enhanced sampling strategies together with simulations under force could provide an appealing approach to access to kinetic rates currently not accessible in unperturbed simulations,¹⁰¹ while working at experimentally relevant forces. This was very recently achieved on model systems while this article was in preparation, which offers very exciting perspectives for the field.¹⁰²

Conclusions and perspectives

We have discussed here some key recent advances and emerging challenges in the field of molecular modeling of mechanical processes in biology. Following the evolution of simulation techniques, model accuracy, and computational power, scientists have been able to

tackle systems of increasing size and complexity, and could recently close the gap between the amplitude of the perturbations used *in silico* as compared to *in vitro* and *in vivo* experimental studies. From the experimental perspective, the concomitant development of new techniques, especially at the single-molecule level, are providing more and more evidence of the importance of force in a wide range of contexts, and of the intimate details of the biomolecular interactions involved in the mechanosensing abilities of cells. Because they can provide molecular details that are often not directly accessible in the experiments, molecular simulations performed at various scales have a key role to play in the interpretation of experimental results. They are now routinely used, in particular as part of an increasing number of joint simulation/experimental studies.

Despite great progress, simulations still face important technical challenges. In particular, and as recently argued,¹⁰⁰ we believe that enhanced sampling techniques could contribute to the exploration of free-energy landscape and kinetics under perturbations very similar to the experimental ones. Improvements in coarse-grained and multiscale approaches⁹⁵ also enables the study of systems and questions at larger scale, or in conditions that are not easily modelled using atomistic MD (such as the application of shear-flow).

The field of mechanobiology thus appears as a stimulating and exciting environment in which theoretical and computational biophysicists and biochemists will continue to play an important role in the years to come.

Acknowledgement

This work was supported by the "Initiative d'Excellence" program from the French State (Grant "DYNAMO", ANR-11-LABX-0011-01). I am also grateful for many experimental collaborations developed over the years on this topic, and for all the scientific discussions that resulted from these interactions. I am particularly thankful to Sergi Garcia-Manyes, Julio Fernandez, Emmanuel Farge, David Giganti and Ronen Berkovich. I also thank collaborators

and coworkers from the computational field, Bruce Berne and Fabio Sterpone, as well as students involved in some of the computational investigations discussed here. I thank Olivier Languin-Cattöen for his help in preparing some of the figures and his work on the FimH systems. I thank Sergi Garcia-Manyes, Fabio Sterpone and Glen Hocky for very useful comments on the manuscript.

Biographical sketch



Guillaume Stirnemann was born in France in 1987. After undergraduate studies at the École normale supérieure (ENS) and a Master's thesis with Pablo Debenedetti at Princeton University, he received his Ph.D. (2011) from ENS and Sorbonne University (Paris, France), under the supervision of Damien Laage. He was then a postdoctoral fellow at Columbia University with Bruce Berne and later at the Institut de Biologie Physico-Chimique (IBPC) in Paris with Fabio Sterpone. In 2014, he was recruited as a CNRS researcher at IBPC where the current interests of his group include the stability, the mechanical properties and the reactivity of biomolecules, and transport phenomena in aqueous solutions, with a special emphasis on questions related to the origins of life.

Conflict of interests

The authors have no conflict of interests to declare.

References

- (1) Iskratsch, T.; Wolfenson, H.; Sheetz, M. P. Appreciating force and shape—the rise of mechanotransduction in cell biology. *Nat. Rev. Mol. Cell Biol.* **2014**, *15*, 825–833.
- (2) Freundt, J. K.; Linke, W. A. Titin as a force-generating muscle protein under regulatory control. *J. Applied Physiol.* **2019**, *126*, 1474–1482.
- (3) Sharma, S.; Subramani, S.; Popa, I. Does protein unfolding play a functional role in vivo? *FEBS J.* **2021**, *288*, 1742–1758.
- (4) Springer, T. A. Von Willebrand factor, Jedi knight of the bloodstream. *Blood* **2014**, *124*, 1412–1425.
- (5) Thomas, W. E.; Trintchina, E.; Forero, M.; Vogel, V.; Sokurenko, E. V. Bacterial adhesion to target cells enhanced by shear force. *Cell* **2002**, *109*, 913–923.
- (6) Neuman, K C and Nagy, A. Single-molecule force spectroscopy: optical tweezers, magnetic tweezers and atomic force microscopy. *Nat. Methods* **2012**, *5*, 491–505.
- (7) Roca-Cusachs, P.; Conte, V.; Trepast, X. Quantifying forces in cell biology. *Nat. Cell Biol.* **2017**, *19*, 742–751.
- (8) Mohammed, D.; Versaevel, M.; Bruyère, C.; Alaimo, L.; Luciano, M.; Vercruyssen, E.; Procès, A.; Gabriele, S. Innovative tools for mechanobiology: Unraveling outside-in and inside-out mechanotransduction. *Front. Bioeng. Biotechnol.* **2019**, *7*, 162.
- (9) Freikamp, A.; Cost, A. L.; Grashoff, C. The piconewton force awakens: Quantifying mechanics in cells. *Trends Cell Biol.* **2016**, *26*, 838–847.

- (10) Isralewitz, B.; Gao, M.; Schulten, K. Steered molecular dynamics and mechanical functions of proteins Isralewitz, Gao and Schulten 225. *Curr. Opin. Struct. Biol.* **2001**, *11*, 224–230.
- (11) Franz, F.; Daday, C.; Gräter, F. Advances in molecular simulations of protein mechanical properties and function. *Curr. Opin. Struct. Biol.* **2020**, *61*, 132–138.
- (12) Grubmüller, H.; Heymann, B.; Tavan, P. Ligand binding: Molecular mechanics calculation of the streptavidin-biotin rupture force. *Science* **1996**, *271*, 997–999.
- (13) Lu, H.; Schulten, K. Steered molecular dynamics simulations of force-induced protein domain unfolding. *Proteins: Struct., Funct., Bioinf.* **1999**, *35*, 453–463.
- (14) Makarov, D. E. Perspective: Mechanochemistry of biological and synthetic molecules. *J. Chem. Phys.* **2016**, *144*, 030901.
- (15) Ribas-Arino, J.; Marx, D. Covalent mechanochemistry: theoretical concepts and computational tools with applications to molecular nanomechanics. *Chem. Rev.* **2012**, *112*, 5412–5487.
- (16) Cossio, P.; Hummer, G.; Attila, A. On artifacts in single-molecule force spectroscopy. *Proc. Natl. Acad. Sci. U.S.A.* **2015**, *112*, 14248–14253.
- (17) Berkovich, R.; Hermans, R. I.; Popa, I.; Stirnemann, G.; Garcia-Manyes, S.; Berne, B. J.; Fernandez, J. M. Rate limit of protein elastic response is tether dependent. *Proc. Natl. Acad. Sci. U.S.A.* **2012**, *109*, 14416–14421.
- (18) Nam, G. M.; Makarov, D. E. Extracting intrinsic dynamic parameters of biomolecular folding from single-molecule force spectroscopy experiments. *Protein Sci.* **2016**, *25*, 123–134.
- (19) Célerse, F.; Lagardère, L.; Derat, E.; Piquemal, J. P. Massively parallel implementation of steered molecular dynamics in Tinker-HP: Comparisons of polarizable and

- non-polarizable simulations of realistic systems. *J. Chem. Theo. Comput.* **2019**, *15*, 3694–3709.
- (20) Rico, F.; Russek, A.; González, L.; Grubmüller, H.; Scheuring, S. Heterogeneous and rate-dependent streptavidin–biotin unbinding revealed by high-speed force spectroscopy and atomistic simulations. *Proc. Natl. Acad. Sci. U.S.A.* **2019**, *116*, 6594–6601.
- (21) Rico, F.; Gonzalez, L.; Casuso, I.; Puig-Vidal, M.; Scheuring, S. High-speed force spectroscopy unfolds titin at the velocity of molecular dynamics simulations. *Science* **2013**, *342*, 741–743.
- (22) Dudko, O. K.; Hummer, G.; Szabo, A. Theory, analysis, and interpretation of single-molecule force spectroscopy experiments. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 15755–15760.
- (23) Bullerjahn, J. T.; Sturm, S.; Kroy, K. Theory of rapid force spectroscopy. *Nat. Commun.* **2014**, *5*, 4463.
- (24) Sheridan, S.; Gräter, F.; Daday, C. How fast Is too fast in force-probe molecular dynamics simulations? *J. Phys. Chem. B* **2019**, *123*, 3658–3664.
- (25) Stirnemann, G.; Giganti, D.; Fernandez, J. M.; Berne, B. J. Elasticity, structure, and relaxation of extended proteins under force. *Proc. Natl. Acad. Sci. U.S.A* **2013**, *110*, 3847–52.
- (26) Perales-Calvo, J.; Giganti, D.; Stirnemann, G.; Garcia-Manyes, S. The force-dependent mechanism of DnaK-mediated mechanical folding. *Sci. Adv.* **2018**, *4*, eaaq0243.
- (27) Guerin, M. E.; Stirnemann, G.; Giganti, D. Conformational entropy of a single peptide controlled under force governs protease recognition and catalysis. *Proc. Natl. Acad. Sci. U.S.A* **2018**, *115*, 11525–11530.

- (28) Booth, J. J.; Shalashilin, D. V. Fully Atomistic Simulations of Protein Unfolding in Low Speed Atomic Force Microscope and Force Clamp Experiments with the Help of Boxed Molecular Dynamics. *J. Phys. Chem. A* **2016**, *120*, 700–708.
- (29) Mücksch, C.; Urbassek, H. M. Accelerating steered molecular dynamics: Toward smaller velocities in forced unfolding simulations. *J. Chem. Theo. Comput.* **2016**, *12*, 1380–1384.
- (30) Hartmann, M. J.; Singh, Y.; Vanden-Eijnden, E.; Hocky, G. M. Infinite switch simulated tempering in force (FISST). *J. Chem. Phys.* **2020**, *152*, 244120.
- (31) Beedle, A. E.; Lezamiz, A.; Stirnemann, G.; Garcia-Manyes, S. The mechanochemistry of copper reports on the directionality of unfolding in model cupredoxin proteins. *Nat. Commun.* **2015**, *6*, 1–9.
- (32) Stirnemann, G.; Sterpone, F. Mechanics of protein adaptation to high temperatures. *J. Phys. Chem. Lett.* **2017**, *8*, 5884–5890.
- (33) Languin-Cattoën, O.; Melchionna, S.; Derreumaux, P.; Stirnemann, G.; Sterpone, F. Three weaknesses for three perturbations: Comparing protein unfolding under shear, force, and thermal stresses. *J. Phys. Chem. B* **2018**, *122*, 11922–11930.
- (34) Röper, J. C.; Mitrossilis, D.; Stirnemann, G.; Waharte, F.; Brito, I.; Fernandez-Sanchez, M. E.; Baaden, M.; Salamero, J.; Farge, E. The major β -catenin/E-cadherin junctional binding site is a primary molecular mechano-transducer of differentiation in vivo. *eLife* **2018**, *7*, e33381.
- (35) Goktas, M.; Luo, C.; Sullan, R. M. A.; Bergues-Pupo, A. E.; Lipowsky, R.; Vila Verde, A.; Blank, K. G. Molecular mechanics of coiled coils loaded in the shear geometry. *Chem. Sci.* **2018**, *9*, 4610–4621.

- (36) Bergues-Pupo, A. E.; Blank, K. G.; Lipowsky, R.; Vila Verde, A. Trimeric coiled coils expand the range of strength, toughness and dynamics of coiled coil motifs under shear. *Phys. Chem. Chem. Phys.* **2018**, *20*, 29105–29115.
- (37) Daday, C.; Kolšek, K.; Gräter, F. The mechano-sensing role of the unique SH3 insertion in plakin domains revealed by Molecular Dynamics simulations. *Sci. Rep.* **2017**, *7*, 1–10.
- (38) Bauer, M. S.; Baumann, F.; Daday, C.; Redondo, P.; Durner, E.; Jobst, M. A.; Milles, L. F.; Mercadante, D.; Pippig, D. A.; Gaub, H. E. et al. Structural and mechanistic insights into mechanoactivation of focal adhesion kinase. *Proc. Natl. Acad. Sci. U.S.A.* **2019**, *116*, 6766–6774.
- (39) Wang, Y. J.; Rico-Lastres, P.; Lezamiz, A.; Mora, M.; Solsona, C.; Stirnemann, G.; Garcia-Manyes, S. DNA binding induces a nanomechanical switch in the RRM1 domain of TDP-43. *J. Phys. Chem. Lett.* **2018**, *9*, 3800–3807.
- (40) Carrion-Vazquez, M.; Oberhauser, A. F.; Fowler, S. B.; Marszalek, P. E.; Broedel, S. E.; Clarke, J.; Fernandez, J. M. Mechanical and chemical unfolding of a single protein: A comparison. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 3694–3699.
- (41) Graham, T. G.; Best, R. B. Force-induced change in protein unfolding mechanism: Discrete or continuous switch? *J. Phys. Chem. B* **2011**, *115*, 1546–1561.
- (42) Sun, L.; Noel, J. K.; Sulkowska, J. I.; Levine, H.; Onuchic, J. N. Connecting thermal and mechanical protein (un)folding landscapes. *Biophys. J.* **2014**, *107*, 2950–2961.
- (43) Tapia-Rojo, R.; Mazo, J. J.; Falo, F. Thermal versus mechanical unfolding in a model protein. *J. Chem. Phys.* **2019**, *151*, 185105.
- (44) Stirnemann, G.; Kang, S. G.; Zhou, R.; Berne, B. J. How force unfolding differs from chemical denaturation. *Proc. Natl. Acad. Sci. U.S.A.* **2014**, *111*, 3413–3418.

- (45) Žoldák, G.; Stigler, J.; Pelz, B.; Li, H.; Rief, M. Ultrafast folding kinetics and cooperativity of villin headpiece in single-molecule force spectroscopy. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, 18156–18161.
- (46) Valle-Orero, J.; Eckels, E.; Stirnemann, G.; Popa, I.; Berkovich, R.; Fernandez, J. M. The elastic free energy of a tandem modular protein under force. *Biochem. Biophys. Res. Commun.* **2015**, *460*, 434.
- (47) Popa, I.; Rivas-Pardo, J. A.; Eckels, E. C.; Echelman, D. J.; Badilla, C. L.; Valle-Orero, J.; Fernández, J. M. A HaloTag anchored ruler for week-long studies of protein dynamics. *J. Am. Chem. Soc.* **2016**, *138*, 10546–10553.
- (48) Yuan, G.; Le, S.; Yao, M.; Qian, H.; Zhou, X.; Yan, J.; Chen, H. Elasticity of the transition state leading to an unexpected mechanical stabilization of titin immunoglobulin domains. *Angew. Chem. Int. Ed. Engl.* **2017**, *56*, 5490–5493.
- (49) Guo, Z.; Hong, H.; Yuan, G.; Qian, H.; Li, B.; Cao, Y.; Wang, W.; Wu, C. X.; Chen, H. Hidden intermediate state and second pathway determining folding and unfolding dynamics of GB1 protein at low forces. *Phys. Rev. Lett.* **2020**, *125*, 198101.
- (50) Schönfelder, J.; De Sancho, D.; Berkovich, R.; Best, R. B.; Muñoz, V.; Perez-Jimenez, R. Reversible two-state folding of the ultrafast protein gpW under mechanical force. *Comm. Chem.* **2018**, *1*, 1–9.
- (51) Mondal, B.; Thirumalai, D.; Reddy, G. Energy landscape of ubiquitin is weakly multidimensional. *J. Phys. Chem. B* **2021**, *125*, 8682–8689.
- (52) Zhuravlev, P. I.; Hinczewski, M.; Thirumalai, D. Low force unfolding of a single-domain protein by parallel pathways. *J. Phys. Chem. B* **2021**, *125*, 1799–1805.
- (53) Berkovich, R.; Mondal, J.; Paster, I.; Berne, B. J. Simulated force quench dynamics shows GB1 protein is not a two state folder. *J. Phys. Chem. B* **2017**, *121*, 5162–5173.

- (54) Berkovich, R.; Fernandez, V. I.; Stirnemann, G.; Valle-Orero, J.; Fernández, J. M. Segmentation and the entropic elasticity of modular proteins. *J. Phys. Chem. B* **2018**, *9*, 4707–4713.
- (55) Huber, D.; Oskooei, A.; Casadevall Solvas, X.; Andrew Demello,; Kaigala, G. V. Hydrodynamics in Cell Studies. *Chem. Rev.* **2018**, *118*, 2042–2079.
- (56) Wioland, H.; Suzuki, E.; Cao, L.; Romet-Lemonne, G.; Jegou, A. The advantages of microfluidics to study actin biochemistry and biomechanics. *J. Muscle Res. Cell. Motil.* **2020**, *41*, 175–188.
- (57) Jaspe, J.; Hagen, S. J. Do protein molecules unfold in a simple shear flow? *Biophys. J.* **2006**, *91*, 3415–3424.
- (58) Dobson, J.; Kumar, A.; Willis, L. F.; Tuma, R.; Higazi, D. R.; Turner, R.; Lowe, D. C.; Ashcroft, A. E.; Radford, S. E.; Kapur, N. et al. Inducing protein aggregation by extensional flow. *Proc. Natl. Acad. Sci. U.S.A.* **2017**, *114*, 4673–4678.
- (59) Bekard, I. B.; Asimakis, P.; Bertolini, J.; Dunstan, D. E. The effects of shear flow on protein structure and function. *Biopolymers* **2011**, *95*, 733–745.
- (60) Chen, Z.; Lou, J.; Zhu, C.; Schulten, K. Flow-induced structural transition in the β -switch region of glycoprotein Ib. *Biophys. J.* **2008**, *95*, 1303–1313.
- (61) Hakala, T. A.; Yates, E. V.; Challa, P. K.; Toprakcioglu, Z.; Nadendla, K.; Matak-Vinkovic, D.; Dobson, C. M.; Martínez, R.; Corzana, F.; Knowles, T. P. et al. Accelerating reaction rates of biomolecules by using shear stress in artificial capillary systems. *J. Am. Chem. Soc.* **2021**, *143*, 16401–16410.
- (62) Szymczak, P.; Cieplak, M. Proteins in a shear flow. *J. Chem. Phys.* **2007**, *127*, 155106.
- (63) Posch, S.; Aponte-Santamaría, C.; Schwarzl, R.; Karner, A.; Radtke, M.; Gräter, F.;

- Obser, T.; König, G.; Brehm, M. A.; Gruber, H. J. et al. Mutual A domain interactions in the force sensing protein von Willebrand factor. *J. Struct. Biol.* **2017**, *197*, 57–64.
- (64) Sterpone, F.; Derreumaux, P.; Melchionna, S. Protein simulations in fluids: Coupling the OPEP coarse-grained force field with hydrodynamics. *J. Chem. Theo. Comput.* **2015**, *11*, 1843–1853.
- (65) Languin-cattoën, O.; Laborie, E.; Yurkova, D. O.; Melchionna, S.; Derreumaux, P.; Belyaev, A. V.; Sterpone, F. Exposure of von Willebrand factor cleavage site in A1A2A3-fragment under extreme hydrodynamic shear. *Polymers* **2021**, *13*, 3912.
- (66) Dickson, A.; Maienschein-Cline, M.; Tovo-Dwyer, A.; Hammond, J. R.; Dinner, A. R. Flow-dependent unfolding and refolding of an RNA by nonequilibrium umbrella sampling. *J. Chem. Theo. Comput.* **2011**, *7*, 2710–2720.
- (67) Radtke, M.; Lippok, S.; Rädler, J. O.; Netz, R. R. Internal tension in a collapsed polymer under shear flow and the connection to enzymatic cleavage of von Willebrand factor. *Eur. Phys. J. E* **2016**, *39*, 32.
- (68) Löf, A.; Müller, J. P.; Brehm, M. A. A biophysical view on von Willebrand factor activation. *J. Cell. Physiol.* **2018**, *233*, 799–810.
- (69) Fu, H.; Jiang, Y.; Yang, D.; Scheiflinger, F.; Wong, W. P.; Springer, T. A. Flow-induced elongation of von Willebrand factor precedes tension-dependent activation. *Nat. Commun.* **2017**, *8*, 324.
- (70) Schneider, S. W.; Nuschele, S.; Wixforth, A.; Gorzelanny, C.; Alexander-Katz, A.; Netz, R. R.; Schneider, M. F. Shear-induced unfolding triggers adhesion of von Willebrand factor fibers. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 7899–7903.
- (71) Zhang, X.; Halvorsen, K.; Zhang, C. Z.; Wong, W. P.; Springer, T. A. Mechanoenzy-

- matic cleavage of the ultralarge vascular protein von Willebrand factor. *Science* **2009**, *324*, 1330–1334.
- (72) Lou, J.; Zhu, C. Flow induces loop-to- β -hairpin transition on the β -switch of platelet glycoprotein Ib α . *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 13847–13852.
- (73) Interlandi, G.; Thomas, W. The catch bond mechanism between von Willebrand factor and platelet surface receptors investigated by molecular dynamics simulations. *Proteins* **2010**, *78*, 2506–2522.
- (74) Aponte-Santamaría, C.; Huck, V.; Posch, S.; Bronowska, A. K.; Grässle, S.; Brehm, M. A.; Obser, T.; Schneppenheim, R.; Hinterdorfer, P.; Schneider, S. W. et al. Force-sensitive autoinhibition of the von Willebrand factor is mediated by interdomain interactions. *Biophys. J.* **2015**, *108*, 2312–2321.
- (75) Kushchenko, Y. K.; Belyaev, A. V. Effects of hydrophobicity, tethering and size on flow-induced activation of von Willebrand factor multimers. *J. Theo. Biol.* **2020**, *485*, 110050.
- (76) Litvinov, R. I.; Kononova, O.; Zhmurov, A.; Marx, K. A.; Barsegov, V.; Thirumalai, D.; Weisel, J. W. Regulatory element in fibrin triggers tension-activated transition from catch to slip bonds. *Proc. Natl. Acad. Sci. U.S.A.* **2018**, *115*, 8075–8080.
- (77) Thomas, W. Catch bonds in adhesion. *Annu. Rev. Biomed. Eng.* **2008**, *10*, 39–57.
- (78) Le Trong, I.; Aprikian, P.; Kidd, B. A.; Forero-Shelton, M.; Tchesnokova, V.; Rajagopal, P.; Rodriguez, V.; Interlandi, G.; Klevit, R.; Vogel, V. et al. Structural basis for mechanical force regulation of the adhesin FimH via finger trap-like β Sheet twisting. *Cell* **2010**, *141*, 645–655.
- (79) Sauer, M. M.; Jakob, R. P.; Eras, J.; Baday, S.; Eris, D.; Navarra, G.; Bernèche, S.;

- Ernst, B.; Maier, T.; Glockshuber, R. Catch-bond mechanism of the bacterial adhesin FimH. *Nat. Commun.* **2016**, *7*, 10738.
- (80) Liu, J.; Amaral, L. A.; Keten, S. Conformational stability of the bacterial adhesin, FimH, with an inactivating mutation. *Proteins: Struct., Funct., Bioinf.* **2021**, *89*, 276–288.
- (81) Interlandi, G.; Thomas, W. E. Mechanism of allosteric propagation across a β -sheet structure investigated by molecular dynamics simulations. *Proteins: Struct., Funct., Bioinf.* **2016**, *84*, 990–1008.
- (82) Aprikian, P.; Interlandi, G.; Kidd, B. A.; Le Trong, I.; Tchesnokova, V.; Yakovenko, O.; Whitfield, M. J.; Bullitt, E.; Stenkamp, R. E.; Thomas, W. E. et al. The bacterial fimbrial tip acts as a mechanical force sensor. *PLoS Biol.* **2011**, *9*, e1000617.
- (83) Alonso-Caballero, A.; Schönfelder, J.; Poly, S.; Corsetti, F.; De Sancho, D.; Artacho, E.; Perez-Jimenez, R. Mechanical architecture and folding of E. coli type 1 pilus domains. *Nat. Commun.* **2018**, *9*, 2758.
- (84) Polacheck, W. J.; Chen, C. S. Measuring cell-generated forces: A guide to the available tools. *Nat. Methods* **2016**, *13*, 415–423.
- (85) Hu, X.; Margadant, F. M.; Yao, M.; Sheetz, M. P. Molecular stretching modulates mechanosensing pathways. *Prot. Sci.* **2017**, *26*, 1337–1351.
- (86) Fernandez-Sanchez, M. E.; Brunet, T.; Röper, J. C.; Farge, E. Mechanotransduction’s impact on animal development, evolution, and tumorigenesis. *Annu. Rev. Cell Dev. Biol.* **2015**, *31*, 373–397.
- (87) Cain, N. E.; Jahed, Z.; Schoenhofen, A.; Valdez, V. A.; Elkin, B.; Hao, H.; Harris, N. J.; Herrera, L. A.; Woolums, B. M.; Mofrad, M. R. et al. Conserved SUN-KASH

- interfaces mediate LINC complex-dependent nuclear movement and positioning. *Curr. Biol.* **2018**, *28*, 3086–3097.
- (88) Jahed, Z.; Hao, H.; Thakkar, V.; Vu, U. T.; Valdez, V. A.; Rathish, A.; Tolentino, C.; Kim, S. C.; Fadavi, D.; Starr, D. A. et al. Role of KASH domain lengths in the regulation of LINC complexes. *Mol. Biol. Cell* **2019**, *30*, 2076–2086.
- (89) Gillespie, P. G.; Müller, U. Mechanotransduction by hair cells: models, molecules, and mechanisms. *Cell* **2009**, *139*, 33.
- (90) Huang, D. L.; Bax, N. A.; Buckley, C. D.; Weis, W. I.; Dunn, A. R. Vinculin forms a directionally asymmetric catch bond with F-actin. *Science* **2017**, *357*, 703.
- (91) Barrick, S.; Li, J.; Kong, X.; Ray, A.; Tajkhorshid, E.; Leckband, D. Salt bridges gate β -catenin activation at intercellular junctions. *Mol. Biol. Cell* **2018**, *29*, 111–122.
- (92) Hytönen, V. P.; Vogel, V. How force might activate talin’s vinculin binding sites: SMD reveals a structural mechanism. *PLoS Comp. Biol.* **2008**, *4*, e24.
- (93) Araya-Secchi, R.; Neel, B. L.; Sotomayor, M. An elastic element in the protocadherin-15 tip link of the inner ear. *Nat. Commun.* **2016**, *7*, 13458.
- (94) De la Torre, P.; Choudhary, D.; Araya-Secchi, R.; Narui, Y.; Sotomayor, M. A mechanically weak extracellular membrane-adjacent domain induces dimerization of protocadherin-15. *Biophys. J.* **2018**, *115*, 2368–2385.
- (95) Batchelor, M.; Papachristos, K.; Stofella, M.; Yew, Z. T.; Paci, E. Protein mechanics probed using simple molecular models. *Biochim. Biophys. Acta* **2020**, *1864*, 129613.
- (96) Freedman, S. L.; Suarez, C.; Winkelman, J. D.; Kovar, D. R.; Voth, G. A.; Dinner, A. R.; Hocky, G. M. Mechanical and kinetic factors drive sorting of F-actin cross-linkers on bundles. *Proc. Natl. Acad. Sci. U.S.A.* **2019**, *116*, 16192–16197.

- (97) Bidone, T. C.; Skeeters, A. V.; Oakes, P. W.; Voth, G. A. Multiscale model of integrin adhesion assembly. *PLoS Comp. Biol.* **2019**, *15*, e1507077.
- (98) Toan, N. M.; Thirumalai, D. Forced-rupture of cell-adhesion complexes reveals abrupt switch between two brittle states. *J. Chem. Eng. Data* **2018**, *148*, 123332.
- (99) Kononova, O.; Kholodov, Y.; Theisen, K. E.; Marx, K. A.; Dima, R. I.; Ataullakhanov, F. I.; Grishchuk, E. L.; Barsegov, V. Tubulin bond energies and microtubule biomechanics determined from nanoindentation in silico. *J. Am. Chem. Soc.* **2014**, *136*, 17036.
- (100) Gomez, D.; Peña Ccoa, W. J.; Singh, Y.; Rojas, E.; Hocky, G. M. Molecular paradigms for biological mechanosensing. *J. Phys. Chem. B* **2021**, *125*, 12115.
- (101) Tiwary, P.; Parrinello, M. From metadynamics to dynamics. *Phys. Rev. Lett.* **2013**, *111*, 230602.
- (102) Ccoa, W. J. P.; Hocky, G. M. Assessing models of force-dependent unbinding rates via infrequent metadynamics. **2021**, arXiv:2112.03249.

Toc picture

