New and Notable

How Accurate Are Ultrasensitive Biophysical Force Probes?

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Many of us can remember that, 20 years ago, measuring the strength of individual bonds between biomolecules or mechanically probing the inner structure of proteins and nucleic acids were a kind of holy grail that could only be dreamt of because of the recent discovery of the atomic force microscope (1). Since then, the field of single-molecule biomechanics emerged and grew exponentially in terms of number of publications and research teams. Many new force probes were developed or adapted for bioapplications. In particular, among the most widely used techniques, atomic force microscope (AFM), optical tweezers (OT), and the biomembrane force probe (BFP) became very popular (2). These three classes of probes (together with other customized variants) allow us now to detect the rupture of ultraweak bonds with picoNewton force sensitivity. The main differences between AFM, OT, and BFP lie in the instrumental strategy to achieve this goal. For instance, the AFM uses relatively stiff force transducers, but fully exploits the large size (100 μm) and the reflective properties of the levers to detect deflections down to subnanometer sensitivity. In contrast, an OT might be orders-of-magnitude softer ($<$0.01 pN/nm), which then does not demand very high spatial resolution, since the thermal fluctuations in probe positions are set by the ratio k_BT/k_F. Finally, the BFP relies on intermediate and tunable spring constants and on nanometer resolution. All these techniques have their own pros and cons. For example, when OTs are used, local overheating of the biological samples remains a recurrent concern, even in the less damaging infrared spectrum (3). Some more subtle drawbacks of these approaches emerged with the recent advent of dynamic force spectroscopy (4). As theoretically proposed and experimentally validated on a number of different systems, the energy landscapes along the unfolding pathway of single proteins (or the unbinding of receptor-ligand bonds) can be obtained by direct mechanical measurements at various loading rates. To get a complete picture of all conformational transitions and wells, two requirements must be fulfilled: 1), one should be able to measure rupture or unfolding forces over several orders of magnitude (typically between 1 and a few hundreds of pN); and 2), experiments should be carried out over an even more extended range of velocities, since the most probable force follows a slow logarithmic dependence with loading rate. In consequence, when micromechanical tests are performed at high velocities, the contribution of the viscous drag in the measured force derived from transducer deformation or probe displacement might become significant (4). This effect is especially critical for AFM cantilevers, due to their larger size as compared with micron-sized trapped beads. This advantage of OT and BFP over AFM must be counterbalanced by the limited range of forces over which OT and BFP behave as linear springs. For example, the potential well of an OT deviates from harmonicity for bead displacements of the order of the probe size. Similarly, large deformations of the pressurized BFP capsule are expected to give rise to a nonlinear force-deformation relationship. For the sake of analytical simplicity, these trivial considerations are often overlooked in single-molecule force experiments.

More generally, when dealing with measurements of minuscule forces involved in individual bonds, one mostly cares about the obtained force sensitivity. The main goal of the present note is, however, to recall that force precision is also an important issue. If relative force variations are usually sufficient to detect and locate transition barriers in an energy landscape, accurate absolute force values from these high-precision instruments can provide even more detailed information about molecular properties. In this issue, Heinrich and Ounkomol address the specific case of the BFP transducer's precision (5). Their article offers an in-depth analysis for the force-deformation response of a pressurized spherical membrane. Since their aim was to investigate the stiffness of the cell transducer from small to large deformations, they started with performing numerical calculations based on classical variational treatment. The surprise came because the exact numerical results were found to deviate significantly from the analytical approximation used so far by all BFP users, even at small deformation. These findings were confirmed by unprecedentedly precise experiments on pressurized red blood cells. The authors finally derived an analytical expression for small deformations, which can thus be used directly for on-line force measurements at the single-molecule level. Previous misestimates of the BFP spring constant certainly do not question the wealth of findings obtained with this technique during the last decade. Still, knowing the exact pressure-dependent stiffness of the capsule and how to correct previous results is a notable improvement in our ability to resolve molecular properties.

For this, and also for the comprehensive understanding of the mechanics of a swollen membrane deformation provided in the article by Heinrich and Ounkomol, I think that this rigorous work may be the first step toward a new way of designing and using force probes in biophysics. It changes

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our focus, which up until now has been on force sensitivity as the main technical challenge in single-molecule experiments, and prompts us to consider more carefully the accuracy of force measurement and its implications. While many receptor-ligand bonds have been studied separately by various techniques, it is now time to collect all the data to build, for instance, an overall picture of the possible weak spots in a chain of bonds in series. To do so, it is necessary to have access to absolute and precise force measurements. Further, if the ultimate goal of all these single-molecule DFS experiments is to yield a molecular basis for a bottom-up approach toward a continuum mesoscopic description of cell mechanics and kinetics, we will need force transducers that work over a large range of forces. This implies that we go far beyond the small-deformation regime of current force probes. Clearly, Heinrich and Ounkomol's work should stimulate other AFM or OT experts to have a look into all these primarily technical issues, which may bring us closer to a profound understanding of the cooperative behavior of clustered individual bonds in cell adhesion, migration, or division.

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