

Preface

The concept of force spectroscopy is deceptively simple: if we could attach a pair of handles to two interacting molecules and use those handles to pull the molecules apart, then we could not only obtain a clear and unambiguous value of the bond strength, but also obtain this value with a very direct and straightforward measurement. People have used the “tug test” to measure and compare strength in many forms throughout history and occasionally this strength testing could take the entertaining form of a “tug-of-war” contest, such as the Japanese *tsunahiki*. Tug-of-war even used to be an Olympic sport in the beginning of the twentieth century. We all have certainly played with the force-measuring springs in a science class at school. So what could be simpler than pulling two species apart?

At least that was my impression when I first got exposed to the concept of using tiny springs to probe molecular-scale interactions. At the time, I was a very green and moderately scared first year graduate student at Harvard who, along with the rest of my classmates, was looking for a research project and for a research group to call home for the next several years. The choice process consisted mostly of listening to gossip, going to each group’s open house, and then gossiping more. Although the conversations mostly revolved around the free pizza typically served at those functions, a good deal of the professors’ research presentations was also discussed. I still remember being very impressed by two events at the open house of Charles Lieber’s group. First, instead of the perfectly respectable but boring pizza, the Lieber group served a lavish Chinese takeout buffet, which was met with considerable delight by everyone in attendance. Second, the research presentation mentioned a project where someone would try to attach specific molecules to the needle of an AFM probe and then use specific interactions of those molecules to perform chemically specific imaging at that unprecedentedly small scale. The concept, which later would become known as chemical force microscopy, had a strange appeal to me, which I immediately shared with my classmate sitting on the conference room floor next to me. His response had a noticeable sarcastic overtone: “Good luck doing it, it sounds complicated. . . .” Like any good advice, it went unheeded, and after a few days of deliberations I signed on. Thus my decade-long fascination with force spectroscopy and its applications to the study of chemical and biological interactions started. Today I would freely admit that the advice I received from my classmate was quite sound. Over the past decade, it has been indeed fascinating to watch researchers uncover an incredibly rich universe of different physical behaviors that originate from such a conceptually simple setup.

Why do researchers continue to be interested in interaction forces when everything we study in the physics, chemistry, and biophysics courses almost always revolves around interaction energies and interaction potentials? Part of the answer lies in the ubiquitous role the interaction forces play in the majority of condensed phase phenomena. These interactions ultimately shape the dynamics of the molecular behavior on the microscopic scale, and direct probing of interaction forces is important for compiling the full picture of these phenomena. Often these processes, most notably in biological systems, involve spectacular

rearrangements and movements of ions, molecules, or whole molecular assemblies driven by mechanical stresses generated by molecular-scale motors. Direct probing of the forces generated by these sophisticated biological machines provides invaluable information about the nature of these processes, and force spectroscopy techniques have been at the forefront of molecular motor studies. Perhaps the most powerful argument for the utility of force spectroscopy techniques is that they provide researchers with a “handle” that they can use to deform the potential energy landscape in the direction of the applied force. Such deformation invariably modifies the kinetics of the molecular bond rupture, and monitoring of the rupture kinetics as a function of the applied force (and force direction) gives us a unique opportunity to study the potential energy landscape of the interactions, often in one direction at a time.

A force spectroscopy measurement almost always involves attaching interacting molecules to a force transducer and then using a mechanical translation device, such as a piezoelectric scanner, to move one of the interacting molecules. In practice, this scheme can be implemented using a large number of very distinct technical approaches. Three of them tend to dominate the force spectroscopy field nowadays. The surface forces apparatus uses ultra-smooth crossing cylinder sheets to probe the interactions between monolayers of interacting species attached to the surfaces of the interacting sheets. Optical and magnetic trapping techniques, which are widely known as “molecular tweezers” techniques, use optical gradients of magnetic fields to trap and move tiny particles or beads. Researchers can use a well-developed arsenal of chemical and biochemical methods to tether different configurations of molecules to the bead surfaces, use the trap to manipulate the beads, and then use highly controlled small forces to study the interaction dynamics. Finally, perhaps the most widespread technique involves using tiny atomic force microscope probes to measure interaction forces between molecules attached to the surfaces of the cantilever tip and the sample.

Each of these measurements addresses several common questions and challenges. First, researchers need to design the experiment to enable probing of a certain specific interactions while discriminating against the non-specific interactions that are always present in real measurements. Second, more often than not, force spectroscopy measurements happen away from equilibrium; therefore researchers need to pay attention to the kinetics of the loading and rupture process and use this information to reconstruct the underlying potential energy landscape of the intermolecular bond. Third, manipulating single molecules on the nanometer scale is rarely precise and researchers are always facing the challenge of estimating properly the number of interacting molecules and relating that to the measured forces.

This book is not intended as a mere survey of the force spectroscopy achievements over nearly two decades of the field’s existence, as such surveys are almost always incomplete in an actively developing field. Instead, the intent is to present a series of topics that discuss fundamental concepts and basic methodology used to perform and understand force spectroscopy experiments and illustrate them using examples from current and past research. Thus the ideal audience that we have imagined for this book is a graduate student who is just starting in the force spectroscopy field and is looking to learn the ropes, or a researcher from an adjacent field who wants to get up to speed with force spectroscopy measurements, or simply wants to evaluate the potential benefit of the technique for her research. Our hope is that this audience will be served well by the material presented in this handbook.

D. Leckband starts the volume by describing the basic principles of the surface forces apparatus measurements and their applications for studies of the protein-protein interactions. C. Lieber, A. Noy, and D. Vezenov give a detailed description of chemical force microscopy—the technique for probing intermolecular interactions using AFM tips functionalized with specific chemical functional groups. R. Conroy presents an extensive survey of the force

measurements using magnetic and optical tweezers—the technique that in many aspects is complementary to the AFM- and SFA-based measurements.

One of the major advancements in force spectroscopy in the last decade has been the emergence of the kinetic model of the bond strength, which caused a paradigm shift in the interpretation of force spectroscopy experiments and spawned the development of dynamic force spectroscopy. A chapter by P. Williams discusses dynamic force spectroscopy and its applications to the AFM experiments. A contribution by K. Anderson, D. Brockwell, S. Radford, and D. A. Smith describes elegant experiments that use dynamic force spectroscopy to probe protein structure.

Functionalization of the force probes with biological molecules is an extremely important part of any force spectroscopy measurement, and the chapter by C. Blancette, A. Loui, and T. Ratto surveys different approaches to functionalization of the force probes. Attaching biomolecules to the force probes via long flexible polymeric tethers has proven to be an extremely versatile, important, and fruitful approach to such functionalization. The chapter by T. Sulchek, R. Friddle, and A. Noy discusses the implementation of this approach, the models used to interpret the results of these measurements, and their application to studies of the strength of multiple bonds. Development of the approaches to probe equilibrium potential energy landscapes of the interactions remains an important goal of the field, and a chapter by P. Ashby describes the design principles and the setup of the AFM measurements that could allow direct reconstruction of this energy landscape. Finally, the continuing explosive growth of the computing power available to researchers brings molecular modeling to the forefront of force spectroscopy research. D. Patrick presents an overview of the modeling of force spectroscopy experiments, with an emphasis on analyzing chemical force microscopy measurements.

I hope that this book will convey a sense that as a result of the last decade of force spectroscopy development our knowledge of the behavior of a non-covalent chemical bond under an external load is immeasurably richer; yet, at the same time, that we now understand the limitations and the complications of the technique with more clarity. The naïve optimism of the first years of force spectroscopy has been replaced with more realistic expectations rooted in the deep understanding of the physical processes underlying the measurements.

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