

Preface

Single-molecule methods have emerged as powerful tools in life science research. These techniques allow the detection and manipulation of individual biological molecules and investigate, with unprecedented resolution, their conformations and dynamics at the nanoscale level. These techniques overcome the restrictions of traditional bulk biochemical studies by focusing not on a population of molecules but on the molecule itself. During the last two decades, this field has grown at an almost exponential rate in terms of biological and biophysical applications. Single-molecule techniques have opened a new field of science, that is, at the crossroads of several disciplines, namely biology, physics, chemistry, material science, and computer science. Since the development of single-ion channel recording techniques in the 1970s, the family of single-molecule methods has expanded significantly to include, among others, optical and magnetic tweezers, atomic force microscopy (AFM), and single-molecule fluorescence.

The present volume considers a range of important and timely applications of biological problems mainly tackled by single-molecule AFM. The AFM was first described in 1986 and originally developed as a high-resolution imaging tool before it began to be used to probe and manipulate molecules. During the last two and half decades, AFM has evolved as a very powerful and versatile tool in biology that can be used, for example, to manipulate and detect single proteins, DNA, or polysaccharides; to image single molecules in physiological conditions with nanometer resolution; to measure the interaction forces between proteins and DNA; to track exocytotic fusion; to map cell surface receptors; or to visualize molecular motors in action. One of the key advantages of the AFM as a single-molecule technique is the straightforward sample preparation, the ability to conduct imaging and manipulation experiments of biomolecules under physiological relevant conditions, and the direct analysis of the dynamics of single molecules or complexes.

The chapters are aimed at the nonexpert—advanced graduate students, postdoctoral investigators or established investigators wanting to enter new fields with the knowledge needed to pursue research in various areas in which single-molecule approaches are important.

Carrión-Vazquez and coworkers contribute with a comprehensive up-to-date review on the use of AFM techniques to study the nano-mechanical properties of structured and disordered proteins. Amyloidogenic proteins are a rich and diverse group of molecules capable of forming amyloid fibrils, and many of them are implicated in severe degenerative diseases. Kellermayer and coworkers describe how single-molecule techniques can be used to study the behavior of amyloidogenic proteins and amyloid fibrils.

Three chapters are devoted to different single-molecule approaches to analyze biomolecular interactions. Hinterdorfer and coworkers discuss single-molecule force spectroscopy as well as simultaneous Topography and RECOgnition imaging (TREC) applied to living cells using AFM techniques. While the force spectroscopy yields information regarding the structure and the dynamics of the recognition process, the TREC method offers the possibility to map the distribution of specific molecular recognition events on the cell surface with nanometer accuracy under physiological condition. Zhu and Chen describe single-molecular interaction experiments that demonstrate catch bonds between integrin receptors and their respective ligands, intercellular adhesion molecule 1, or fibronectin. They also discuss experimental analyses and models that relate integrin catch bonds to their structures and conformations. Lyubchenko outlines the advances in AFM technology in studies of various types of protein–DNA complexes. He gives special attention to time-lapse AFM imaging in aqueous solutions, which enables direct observation of protein–DNA dynamics and interactions.

Ando and coworkers overview the use of high-speed AFM to visualize dynamic events of proteins in action under physiological conditions at subsecond temporal and subnanometer resolution. They highlight recent imaging studies of myosin V walking on an actin filament, rotary catalysis of F_1 -ATPase, and the processive movement of cellulase. Perez-Jimenez and Alegre-Cebollada cover some of the most significant achievements of the single-molecule methods in elucidating enzyme catalysis. They describe recent developments of force-clamp single-molecule assays to study the chemistry of thioredoxin enzymes, a class of oxidoreductases present in all known living organisms that act as electron donors for reducing disulfide bonds.

Alsteens and Dufrêne discuss how single-protein manipulation and imaging experiments can be applied to live cells to shed new light into the highly sophisticated functions of cell surfaces. They review recent progress made in understanding the elasticity, clustering, and functions of cell surface proteins, owing to the integration of the modern tools of molecular genetics (protein design in live cells), with a powerful set of AFM techniques (live cell imaging, single-molecule manipulation, and localization).

In the last chapter, Makarov introduces essential theoretical concepts needed to understand how the dynamics and thermodynamics of proteins are affected by mechanical stress. Various computational approaches and theoretical models used to explore the relationship between protein structure and mechanical response are critically reviewed and computational and theoretical predictions are then contrasted with experimental observations.

I gratefully acknowledge the contributions by the different authors each of whom donated their unique expertise in order to further enhance the ever expanding single-molecule field.

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