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Atomic Force Microscopy

Methods and Protocols

Edited by

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Preface

Atomic force microscopy (AFM) has been applied over the last 30 years in a variety of research fields, including physics, chemistry, engineering, biology, and biomedical sciences. This work intends to collect some of the most relevant and/or recent experimental approaches "using atomic force microscopy in Biology and Biomedical Sciences." Our overall objective was to provide examples of applications using biological samples, showing different methods for AFM sample preparation, data acquisition, and processing and some tips and tricks for optimizing AFM measurements and to avoid problems during them. We have brought together all these recent advances in the AFM field, expecting that this work can be a bibliographic reference for researchers on different stages of know-how working with an AFM in biology, from newcomers with low level of knowledge on the use of this technique to researchers experienced in AFM but that are starting to work with a particular new type of sample, methodology, or data treatment process.

For those researchers interested in studying biological samples using AFM, the availability of a comprehensive source of protocols describing the most recent methodological advances in this technique is invaluable, as many research publications do not provide such detailed information and technical notes that are critical to be successful in developing the experiments. For this reason, we have put together a series of protocols written by a transdisciplinary group of internationally recognized experts working on developing new tools for addressing distinct biological questions, therefore providing guidelines for better performing AFM imaging and force spectroscopy experiments.

The book has 21 chapters, divided into 2 main parts. The first part includes six chapters addressing the AFM imaging of biological samples; the second part is composed of 15 chapters dedicated to different biological applications and experimental aspects of AFM-based force spectroscopy measurements.

In Chap. 1, Eaton and Batziou [1] describe different experimental artifacts and technical issues that an AFM user could face while obtaining AFM images. In this chapter, the authors describe different types of image artifacts pointing solutions to avoid them. This chapter is extremely useful to all AFM users, especially to the new ones, whom have little chance of understanding if something is going wrong with an image. In Chap. 2, Connell et al. explain different methods to process and quantitatively analyze AFM images of phase-separated supported lipid bilayers [2]. In Chap. 3, Nasrallah et al. detail the protocol to fabricate supported lipid bilayers, as well as the main guidelines for successfully using high-speed AFM imaging [3]. Senapati and Park outline the AFM procedures for imaging membrane proteins (rhodopsin nanodomains) and to perform their quantitative analysis in Chap. 4 [4]. A detailed description of the methods to prepare and image DNA-protein complexes is given in Chap. 5 by Pisano and Gilson [5]. The first part of the book ends with Chap. 6, in which Pi and Cai introduce AFM cell topography, which includes the basic principle of AFM imaging, basic operation modes, imaging of biological sample, critical tips for cell topography and its quantitative imaging, as well as some applications [6].

The second part of the book shows different examples of single-molecule force spectroscopy studies and protocols to carry them out. To prepare the samples to perform these studies, first it is necessary to functionalize the AFM tips and supports for molecular recognition. Ebner et al., in Chap. 7, describe a set of methods by which a variety of

proteins, oligonucleotides, or small molecules can be tethered to silicon (nitride) tips or to mica [7]. Ligand-receptor recognition can be studied using AFM-based single-molecule dynamic force spectroscopy. In Chap. 8, Liu et al. describe an example of applying single-molecule dynamic force spectroscopy to study the binding of epidermal growth factor (EGF) to its receptor (EGFR), testing the effect of two clinical drugs on this ligand-receptor interaction [8]. On the same field, Sumbul and Rico, in Chap. 9, provide protocols precisely explaining how to prepare the samples and analyze and interpret the force spectroscopy results in terms of available theories [9]. They also present some molecular dynamics simulations, focusing on steered molecular dynamics that are being used to explore the mechanics of biomolecular processes such as unbinding and unfolding, at the single-molecule level. These authors show the importance of bridging computational tools with the AFM experimental technique. Chapters 10 and 11 are two examples of the application of AFM-based force spectroscopy. In Chap. 10, Unsay and García-Sáez show how to study the effect of pore-forming proteins in supported lipid bilayers [10], while in Chap. 11, Pires et al. set different protocols to study neutrophil extracellular traps using atomic force microscopy [11].

AFM also provides ideal conditions for nanoscale structural and mechanical characterization of bacterial and viral surfaces, on their native and physiological conditions. Four different examples of these studies are described on the next chapters, namely, (a) the protocols by Oh and Hinterdorfer to study bacterial curli production and adhesion (Chap. 12) [12], (b) the strategies to probe antimicrobial peptides' action (also applicable to other antibiotic agents) put forward by Domingues et al. (Chap. 13) [13], and the studies of viruses and their protein shells by Guo and Roos [14] and Ortega-Esteban et al. [15] (Chaps. 14 and 15, respectively). Chapter 15 also explains the combination of AFM and fluorescence methodologies to monitor genome release from individual viral shells during mechanical unpacking.

The mechanical properties of biological samples can also be evaluated by AFM, as it combines precise spatial resolution and high force sensitivity. Examples of how to measure the elastic properties of biological samples are detailed in Chaps. 16–18. Bouchonville and Nicolas, in Chap. 16, propose a methodology to treat rigidity measurement data, by fitting parts of the force-indentation curves that correspond to the linear elastic response of the material [16]. In Chap. 17, Hermann Schillers presents a standardized nanomechanical AFM procedure that strongly reduces the variability of results obtained on soft samples, including living cells, by a reliable method to calibrate AFM cantilevers [17]. AFM-based measurements and data analysis of mechanical properties of single cancer cells are presented in Chap. 18 by Lekka and Pabijan [18].

Finally, the last three chapters of this book are dedicated to AFM applications in medicine. Gomes et al., in Chap. 19, describe the use of molecular recognition force spectroscopy for the characterization and optimization of targeting nanoparticles toward a given cell-specific interaction [19]. Chapters 20 and 21 are focused on the biomechanical characterization and activity assessment of live human cardiomyocytes. Pribyl et al., in Chap. 20, describe the construction of an AFM-based biosensor setup designed to study the biomechanical properties of cardiomyocyte clusters [20]. On a related work [21], Caluori et al. studied the single cardiomyocyte electro-chemo-mechanics during excitation-contraction coupling (Chap. 21). They explain in detail how to implement such an in vitro system, which can monitor cardiac electrophysiology, intracellular calcium dynamics, and single-cell mechanics.

In addition to the protocols themselves, the Notes section of each chapter provides extremely useful and interesting information about some tips and tricks that are not typically published in the Methods sections of other standard journal articles.

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Lisbon, Portugal

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