Acoustic Force Spectroscopy (AFS)

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Chapter 8

Summary and Conclusions

Abstract

Biophysics has the daring task to understand the processes governing life in a quantitative manner, combining the fields of Biology, Chemistry and Physics. Given the huge complexity of life processes, biophysicists need to approach a system by looking at different length scales, from single biomolecules up to colonies of organisms. In this thesis, I present a new experimental technique that is able to investigate systems over a wide range of length scales, from single molecules (nm) up to the single cell (μm). The summary below provides a concise overview of the key results presented.

In **Chapter 1** I have given a general introduction of the topic of this thesis. I argued that life is complex with multiple layers of complexity. The smallest units of life are cells, therefore they are called "*the building blocks of life*". Cells consist of millions of biomolecules that behave in stochastic and inhomogeneous manners, giving rise to a high order of complexity. To unravel the behavior of a complex system, many approaches are needed. To this end, I developed a new high-throughput method that can be used at the single-molecule and single-cell levels, allowing to get a deeper understanding of the working mechanisms of the cell.

The core of this new technique, Acoustic Force Spectroscopy (AFS), has been presented in **Chapter 2**. AFS is based on the acoustic manipulation of biomolecules tethering a microsphere to a surface. Two powerful features of this new method are the ability to apply controllable forces ranging from sub-piconewton to hundreds of piconewtons and the possibility to perform measurements in a high-throughput fashion on thousands of biomolecules in parallel. I applied the technique to biologically relevant systems, to show its different capabilities, such as: (1) measurement of force-extension curves of DNA to determine its biomechanical properties, (2) determination of a protein (here RecA) binding and unbinding to a force-clamped DNA, and (3) dynamic force-spectroscopy measurements of the unbinding kinetics of an antibody (here digoxigenin) to its antigen, to investigate their energy landscape. These experiments showed that AFS is an accurate single-molecule technique that can provide insights in the properties of individual biomolecules and their interactions. This makes AFS a new powerful tool in the single-molecule force spectroscopy toolbox. Compared to other force-spectroscopy techniques, AFS distinguishes itself by a high experimental throughput, a wide range of forces that can be applied with sub-millisecond time response, long-time stability and a wide range of force loading rates.

After the introduction of the AFS method, I described in **Chapter 3** a set of advances to improve the usability and the performance of the method. To improve the imaging capabilities, I replaced the opaque piezo element that was originally used to generate the acoustic field inside the sample chamber with a transparent piezo element, without any drawback. Furthermore, I showed that it is possible to use AFS in combination with high NA liquid-immersion objectives, however at the cost of a reduction of the forces that can be generated. These two improvements make the integration of AFS in most existing microscopes substantially easier. Additionally, I developed a modelling tool to optimize the layer thicknesses of the sample chamber, which allowed to realize a new configuration of the layer thickness of the AFS chip

that can generate a high acoustic force on the bottom side of the flow chamber, previously not possible. Finally, I developed a method to experimentally calibrate the acoustic field inside the flow chamber predicted by my model and used that method to quantitatively test the new chip configuration. The efficiency was increased to such an extent that no high-power amplifier was necessary anymore. These technical improvements make AFS more suitable for an even broader range of applications.

AFS permits high experimental throughput on many individual molecules in parallel, with a wide range of forces and force loading rates. However, setting up a single-molecule experiment can be challenging. Therefore, I described in **Chapter 4** in detail how to set up, perform and analyze an AFS measurement to obtain reliable and valuable data, providing guidelines to future users of the technique.

In **Chapter 5** I used AFS to study the formation of virus capsids that self-assemble on DNA. While viruses are the cause of many diseases, they are recently also used as drug delivery systems. Therefore, the investigation of their self-assembly process, which is poorly understood, is crucial. In this chapter, I studied the dynamics of the self-assembly process of a natural virus and an artificial virus-like particle on a DNA tether. Combining my experimental results with measurements made with atomic force spectroscopy and optical tweezers led to a deeper understanding of the self-assembly process of these viruses. These techniques are complementary to each other and AFS is particularly useful to unravel the dynamics of the process, thanks to its long-time stability, its intrinsic force-clamp properties and the possibility to study many molecules in parallel.

In **Chapter 6** I used AFS to resolve the folding free energy landscape of a simple DNA hairpin. The hairpin is a single DNA strand, consisting of two sequence regions (20 bases) complementary to each other. These strands can exist in either a folded or an unfolded conformation. In chapter 2, I showed already that AFS can be used to probe the energy landscape, however, to fully resolve it, many individual measurements have to be performed over a wide range of forces. In **Chapter 6** used the Bell-Evans theory and the Continuous Effective Barrier Approach to map the folding free energy landscape. My results are in close agreement with previous experimental data obtained with optical tweezers on this hairpin, validating my approach. The two main advantages of unraveling the folding energy landscapes of hairpins with AFS are (1) a significant reduction of the time of experimentation thanks to the multiplexing capabilities and (2) the possibility of probing a wider dynamic

force range. These measurements demonstrate the readiness of AFS to map the energy landscape of more complex systems, such as RNA and proteins.

Finally, in **Chapter 7**, I described a new application of the AFS method, where I used the technique to directly apply forces to single cells and measure their adhesion properties. Assessing strength and kinetics of molecular interactions of cells with the extracellular matrix is fundamental to understand cell-adhesion processes. To this end, I functionalized the surface of the flow chamber with fibronectin, to mimic the extracellular matrix and I followed the binding kinetics of hundreds of cells inside the sample chamber. Subsequently, I ramped up the acoustic force, thereby breaking the cell-surface bonds and I determined the rupture force for each individual cell. Using this approach, I resolved the adhesion strength and kinetics of individual T-cells binding to a fibronectin-modified surface and discriminated different behaviors of subsets of cells. I observed that interleukin 7 (a growth factor proposed to facilitate T-cell adhesion) accelerates the binding kinetics, but the adhesion strength remains the same. Activation of these cells with interleukin 7 likely increases their chance to bind to the vessel wall in the blood flow to locally coordinate the immune response. With these measurements, I demonstrated that AFS can be used to manipulate and track hundreds of cells in parallel in real time, while well-controlled forces can be applied up to 1000 pN. Combined with the fact that the measurements are performed in a closed fluidics system and require only small volumes of biological sample, this new approach opens up a wide range of potential research and clinical applications.

In conclusion, this thesis contributes to the field of Biophysics with the establishment of a novel single-molecule force-spectroscopy technique, AFS (**Chapter 2** and **3**). After providing details on how to setup and perform an AFS measurement (**Chapter 4**), I demonstrated the use of AFS on diverse and biologically relevant systems, by performing measurements on virus-capsid binding to DNA (**Chapter 5**) and reconstituting the energy landscape of a DNA hairpin (**Chapter 6**). Finally, I showed a new application of AFS, where acoustic forces are applied directly on living cells (**Chapter 7**). With this work, I made a contribution to field of Biophysics, laying the foundations for more research with AFS. The methodology described in this thesis, combined with the fact that AFS is already commercially available, makes this method widely accessible. Further advances in the method could in the future even result in medical diagnostics applications.

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