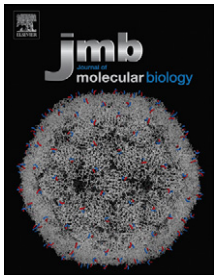
Review



The Power of Force: Insights into the Protein Folding Process Using Single-Molecule Force Spectroscopy

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Abstract

One of the major challenges in modern biophysics is observing and understanding conformational changes during complex molecular processes, from the fundamental protein folding to the function of molecular machines. Single-molecule techniques have been one of the major driving forces of the huge progress attained in the last few years. Recent advances in resolution of the experimental setups, aided by theoretical developments and molecular dynamics simulations, have revealed a much higher degree of complexity inside these molecular processes than previously reported using traditional ensemble measurements. This review sums up the evolution of these developments and gives an outlook on prospective discoveries.

Introduction

The central conundrum of how life evolves at the molecular level sparked questions like how proteins are encoded, how they are synthesized, and how do they acquire their 3D structure in order to fulfill their designated function. Hence, much scientific work has been focused on this type of biomolecule and related biomolecular processes. In particular, the organization of the protein polypeptide chain into its well-defined native form, that is, protein folding, has been one of the grand challenges of modern biophysics ever since [1–3]. An enormous progress has been made, resulting in a wide variety of different experimental techniques including, for example, X-ray crystallography, differential scanning calorimetry, and spectroscopic techniques to answer these questions [4,5]. During the last half-century, it became possible to investigate protein structure, thermodynamics, and kinetics with near atomistic resolution and within corresponding time scales reaching the limit of ns to μs in order to track the dynamics of fast-folding proteins and the formation of secondary structure elements, that is, the roadmap of the folding process [6]. Around 20 years ago, a further milestone was achieved by implementing single-molecule experimental detection and manipulation into modern biophysical research [7–9]. Together with computational science, single-molecule biophysics has become a crucial part of life sciences capable of uncovering biomolecular properties at the nanoscale. Herein, the most recent advances both in single-molecule experiments [10–13] and in molecular dynamics (MD) simulations [3,14,15] have paved the way to understand in-depth molecular processes like the folding of a protein [16] and to describe complex molecular systems, such as the synthesis of the encoded protein by the ribosome [17]. During the last decades, these developments have added an important extension to the traditional ensemble experiments used for the study of the thermodynamics and kinetics of biomolecular systems, including protein folding [1–6]. Nowadays, it is widely accepted that in order to achieve a complete experimental description of any molecular process of interest, at least one single-molecule technique is required, because it provides a detailed description down to the single-molecule level, whereas traditional ensemble experiments would average out discrete dynamic events. It is fair to say that single-molecule techniques have revolutionized the way we study molecular processes, with it being one of the major scientific breakthroughs of the 21st century.

Many, different experimental designs have been developed in order to measure the conformational dynamics of biomolecules at the single-molecular level, which can now cover relevant scales in length (nanometers, nm), force (picoNewtons, pN), and time (microseconds, μs). The variety of experimental setups available is determined by the different physical principles used for the measurements. We can now take advantage of electromagnetic waves {optical tweezers (OT) [18] and Förster resonance energy transfer (FRET) spectroscopy [19]}, a magnetic field (magnetic tweezers [18]) and electric fields (protein nanopore [20,21]), electromechanical signal transduction {atomic force microscope (AFM) [18]}, or recently even acoustic waves (acoustic force spectroscopy [22]). Each of these techniques has its own pros and cons, and the choice of one *versus* the other may depend on the molecular process we want to study and/or the question we want to answer. However, one of the essential differences between the various setups is the nature of the agent used for manipulating the sample molecule, which can be a chemical denaturant (usually, urea or guanidinium chloride), the temperature, or a mechanical force. When focusing on the investigation of protein folding with single-molecule experiments, it is now well understood that the perturbation induced by an external force can produce a different change in the protein free energy landscape (FEL) from that produced by a chemical denaturant as in the case of the src SH3 protein [23] and of the cold shock protein (Csp) [24] compared to the first experiments, where both pertubations can also result in the same FEL description as investigated for the I27 titin domain [25]. Thus, different unfolding behaviors of the same protein could be observed.

The application of an external mechanical force—the hallmark of single-molecule force spectroscopy (SMFS) techniques—has the advantage of probing a clearly defined and controllable reaction coordinate. In the same way that the fraction of native contacts or the RMSD to the native state are used in simulations to monitor folding and unfolding, the distance between protein ends (or other attachment points) is measured and manipulated in single-molecule pulling experiments to monitor the extension of the polymer chain. Furthermore, a mechanical force plays a fundamental role in many biological processes [26,27]. Mechanical processes are involved in many of the biological functions of the cell. In this sense, numerous cases have been described wherein mechanical forces are directly applied to. Among these, we can highlight muscle contraction, cell adhesion, or mechanotransduction, where the proteins involved can either withstand mechanical forces or exert forces ranging from 0.5–10 pN (protein domain motion), 1–100 pN (protein domain deformation) and 50–200 pN (protein domain unfolding) [28]. These scenarios favor the use of SMFS techniques as they can mimic some biological conditions when investigating, for example, protein (un)folding or more complex protein-based molecular systems [29,30]. For example, a stretched unfolded state of a protein, which can just be reached by using a mechanical force, can be observed in the force denaturation of ClpX complex [31]. Thus, we can safely say that, unlike chemical denaturation by urea or guanidinium, force is a biological perturbation commonly encountered in nature.

At the same time, simulations and relevant theories that describe biomolecular processes have seen great developments in the past years. Atomistic [14,15] and coarse-grained molecular simulations [32] have been improved with respect to both their accuracy and the new hardware used for conducting them. Validation against single-molecule experiments can now assist this progress. Recently, atomistic MD simulations reached the experimental time scale of ms [33], and the accuracy of their predictions has been greatly improved due to the optimization of force fields [34,35]. Both experiment and simulation have helped build, verify, and improve existing theories like transition path theory [36–38], Kramers-based theories in the presence of a mechanical force [39–41], or the Crooks fluctuation theorem [42,43] in order to describe complex molecular processes like protein folding [3,44,45]. Recently, single-molecule experiments have been used to validate theoretical predictions for estimating the transition path times of proteins and nucleic acids [46,47].

This review sums up the new developments in the field of SMFS with the focus on AFM and OT techniques. The current progress in both techniques has marked a new episode in modern research. Furthermore, we provide an overview of experimental developments of single-molecule techniques to describe biological systems, together with important recent progress in simulating experimental observations and in developing theory.

Principles of Single-Molecule Experiments

There are two broad categories of single-molecule experiments in relation to the study of protein dynamics (Fig. 1a). On the one hand, we find single-molecule fluorescence spectroscopy using FRET, which can be seen more as an observational technique where the sample is passively followed by the experimentalist, and on the other hand, SMFS studies, which can be regarded as a combination of an observational and probing technique, as it physically has the ability to manipulate the sample.

In the case of single-molecule FRET (smFRET), the emission of photons by a fluorescent donor and acceptor dye is detected. The measured signal depends on the distance between the two labels attached, for example, on the protein of study in order to follow its (un)folding behavior [19]. For the