Available online at www.sciencedirect.com

SciVerse ScienceDirect

Current Opinion in Structural Biology

Force as a single molecule probe of multidimensional protein energy landscapes

Gabriel Žoldák[1] and Matthias Rief[1,2]

Force spectroscopy has developed into an indispensable tool for studying folding and binding of proteins on a single molecule level in real time. Design of the pulling geometry allows tuning the reaction coordinate in a very precise manner. Many recent experiments have taken advantage of this possibility and have provided detailed insight the folding pathways on the complex high dimensional energy landscape. Beyond its potential to provide control over the reaction coordinate, force is also an important physiological parameter that affects protein conformation under *in vivo* conditions. Single molecule force spectroscopy studies have started to unravel the response and adaptation of force bearing protein structures to mechanical loads.

Addresses
[1] Physik Department E22, Technische Universität München, James-Franck-Strasse, 85748 Garching, Germany
[2] Munich Center for Integrated Protein Science, 81377 München, Germany

Corresponding author: Rief, Matthias (mrief@ph.tum.de, Matthias.Rief@mytum.de)

Current Opinion in Structural Biology 2012, **23**:xx–yy

This review comes from a themed issue on **Folding and binding**

Edited by **Jayant Udgaonkar** and **Susan Marqusee**

S0959-440X/$ – see front matter, Published by Elsevier Ltd.

http://dx.doi.org/10.1016/j.sbi.2012.11.007

Introduction

The ultimate goal in experimental studies of protein folding is obtaining three-dimensional structural dynamics with nanosecond time-resolution and large enough dynamic range to sample rare events that only occasionally appears over long time intervals. On the simulation side, all-atom molecular dynamics simulations are already providing full structural dynamics information on ns timescales and recently even ms timescales have been reported [1]. However, no experimental method can currently provide a similarly detailed picture. Single molecule force spectroscopy, while still far from this goal, provides the possibility to measure structural dynamics along a single reaction coordinate with submillisecond resolution over a timescale of minutes, and thus can uncover fine details of the underlying multidimensional energy landscape.

In this review, we will briefly describe the instrumentation and protocols for single molecule force spectroscopy and provide recent examples on how these techniques have yielded insights into protein folding mechanisms. For the interested reader, the stage of the field before 2009 is reviewed in more detail by Puchner and Gaub [2].

Instrumentation and protocols in single-molecule force experiments

AFM and optical tweezers: basic instrumentation for protein mechanics

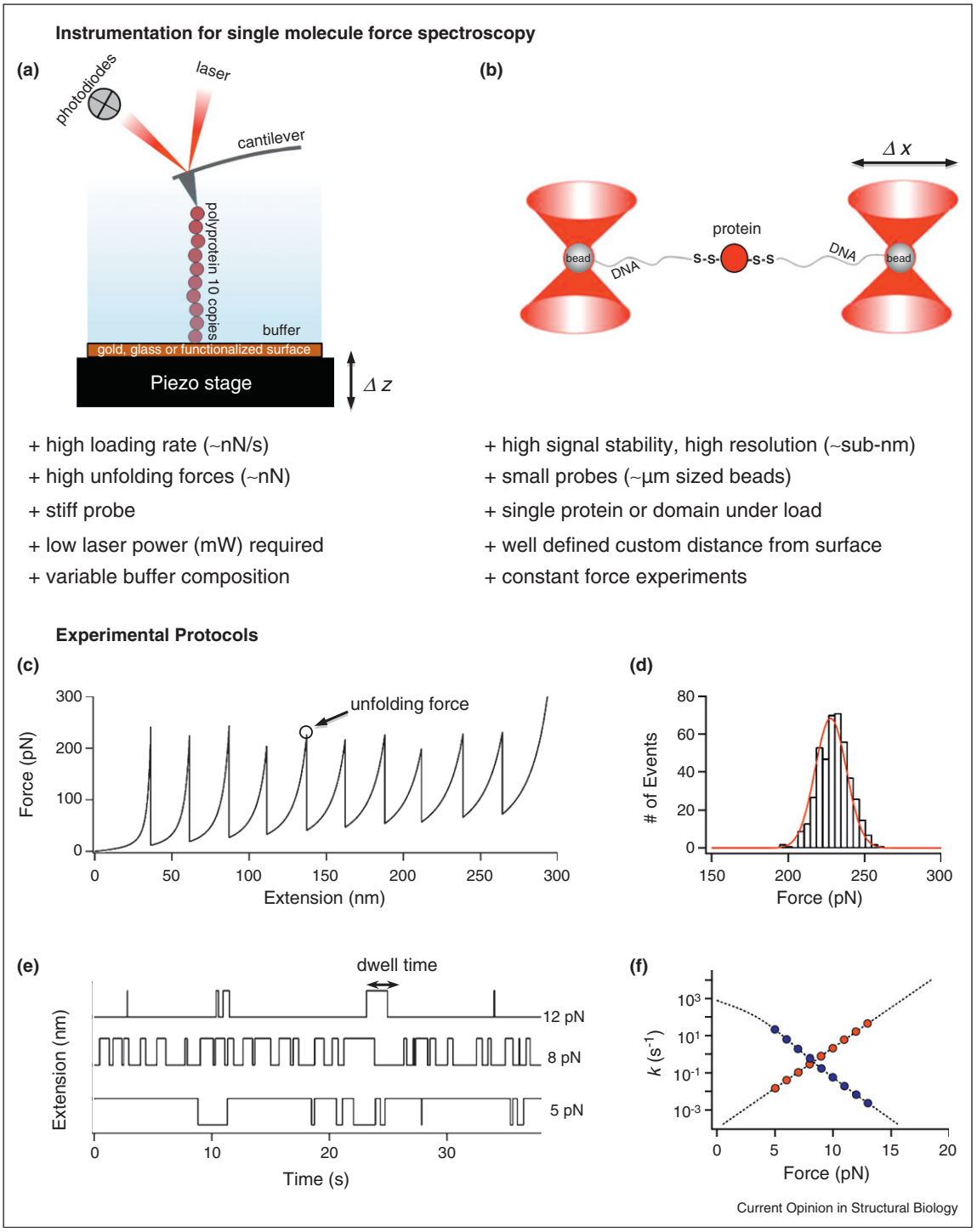
A number of assays that probe the structural response of a single molecule subject to mechanical forces have been developed in the past two decades. The most widely used are atomic force microscopy (AFM) (Figure 1a) and optical tweezers (Figure 1b). The first experiments of mechanical protein unfolding were conducted in 1997 on the muscle protein titin using AFM and optical tweezers [3–5]. In the AFM experiments, it was demonstrated that applying force to short stretches of concatenated polyprotein domains lead to a characteristic single molecule unfolding sawtooth pattern, which emerged as a powerful method to study protein unfolding [3,6] (Figure 1). A few years later, Cecconi *et al.* developed and applied an optical tweezers assay using DNA molecules as covalently bound handles (Figure 1b) that provided enough sensitivity to study the folding of individual ribonuclease H molecules [7]. Both AFM and optical tweezers are now widely used for single molecule force experiments, and they probe complementary force ranges. We will briefly discuss both AFM and optical tweezers experiments as well as summarize their advantages for studying protein mechanics (Figure 1).

AFM is a powerful technique to apply the high forces (>15 pN up to several nN) necessary for unfolding or dissociating stable proteins and protein complexes (Figure 1a). Because of the high spring constant of the cantilever (100–6 pN/nm), AFM provides a fast response that is well-suited to observe transient unfolding intermediates. Refolding rates can be measured and quantified indirectly using double jump force experiments [8]. Both the limited force resolution as well as drift problems on a second time-scale mainly owing to the gold-coated cantilevers often make it difficult to directly observe refolding under load in AFM experiments. However, in a few cases direct observation of equilibrium refolding–unfolding transitions in proteins such as calmodulin [9] and ankyrin [10,11] could be observed also in AFM studies.

Optical traps are ideally suited for the low-to-intermediate force regime (0.5–65 pN), which allows for a detailed study of protein folding pathways. Using a dual trap

Please cite this article in press as: Žoldák G, Rief M. Force as a single molecule probe of multidimensional protein energy landscapes, Curr Opin Struct Biol (2012), http://dx.doi.org/10.1016/j.sbi.2012.11.007

Figure 1



Experimental protocols in single molecule force spectroscopy. **(a)** Atomic force microscope (AFM) used for pulling experiments; immobilized proteins on a gold, glass or any functionalized surface are picked up by the cantilever and stretched at constant velocity by moving of the piezo-stage along the *z*-axis. The position of the cantilever apex is detected by quadrant photodiodes measuring the reflected laser beam. Strengths of the AFM assay are listed below. **(b)** A dual differential setup of a pulling experiment using optical tweezers (for details see [12,61]). The beads are trapped by the foci of two laser beams and the molecular construct is pulled in the direction of the *x*-axis by manipulating one of the beams by a beam steering device (piezo-mirror, AOD, *etc*.). Forces are measured by the deflection of the beads from the trap centers using position sensitive devices. Strengths of the optical tweezers assay are listed below. **(c)** A schematic sample trace of a constant velocity experiments using a polyprotein. When the protein is stretched the individual folded domains in the chain unfold sequentially resulting in the characteristic sawtooth pattern. Each of the peaks reflects the unfolding of a single domain. **(d)** From the maximum of the unfolding peaks, the distribution of the unfolding forces is obtained. The shape and the position of the

Please cite this article in press as: Žoldák G, Rief M. Force as a single molecule probe of multidimensional protein energy landscapes, Curr Opin Struct Biol (2012), http://dx.doi.org/10.1016/j.sbi.2012.11.007