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Single molecule force spectroscopy: a new tool for bioinorganic chemistry

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Metalloproteins are essential in biology. The incorporation of metal ion into metalloproteins significantly expands protein functionality and enhances protein stability. Over the last few years, atomic force microscopy-based single molecule force spectroscopy (SMFS) has evolved into a unique tool allowing for probing metalloproteins and metal–ligand bonds one molecule/bond at a time. Mechanical strength of a wide variety of metal–ligand bonds has been measured in metal–ligand complexes as well as in metalloproteins, providing detailed information of their underlying free energy profiles and the influence of the protein environment on the bond strength. SMFS experiments have directly demonstrated the effect of the metal binding on the mechanical stability of proteins. Moreover, SMFS has enabled the direct observation of the unfolding and folding of metalloproteins, revealing detailed mechanistic insight into the unfolding pathways modulated by the metal center.

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Introduction

Metalloproteins are ubiquitous in nature and play essential roles in a wide variety of biological processes. In metalloproteins, metal ions coordinate with amino acid residues to constitute the metal center, which serves as the enzymatic active center to entail the functionality of proteins or as structural sites to facilitate protein folding and assembly [1]. To understand the structure, function and dynamics of metalloproteins, a suite of methodologies have been developed to tackle specific issues surrounding metalloproteins, and insights obtained using these tools have advanced our understanding of metalloproteins significantly [2–4]. Most of these methodologies provide ensemble average information about the given physical and chemical properties of the metalloprotein/metal center. Moreover, due to the complexity of metalloproteins, developing new tools remains an important task in the field. Over the last two decades, the development of single molecule techniques has provided new tools to probe metalloproteins at the single molecule level, in particular, single molecule fluorescence and single molecule force spectroscopy (SMFS) techniques [5,6]. This article will be focused on SMFS.

Over the last two decades, atomic force microscopy-based SMFS has evolved into a powerful technique in the field of single molecule biophysics and chemistry [7•]. SMFS has enabled the direct measurement of the inter/intramolecular interactions (such as ligand–receptor interaction [8,9] and covalent bonds [10•,11,12]) as well as the mechanical/elastic properties of a wide range of macromolecules, ranging from polysaccharides, DNA, synthetic polymers and all the way to proteins [13••,14–17,18•], at the single molecule level with an unprecedented pico-Newton resolution. These measurements have provided a rich wealth of information about the underlying free energy profiles and molecular mechanisms of these inter/intra-molecular interactions as well as the force-induced conformational transitions in macromolecules, which is otherwise difficult to obtain using traditional ensemble methods. In particular, SMFS has become an indispensable tool to study protein folding-unfolding dynamics as well as protein elasticity at the single molecule level [13••,18•,19,20••]. In combination with steered molecular dynamics simulations [21], SMFS studies have provided detailed mechanistic insights into the protein folding-unfolding mechanism, including those under the influence of ligand binding, as well as the elastic properties of elastomeric proteins and their regulation. Excellent reviews have been published to cover these diverse topics of SMFS and interested readers are referred to these reviews [14,16,17,22].

Here we will provide a brief review of the use of SMFS to probe the metal–ligand interactions, and the folding-unfolding dynamics of metalloproteins at the single molecule level. Compared with the extensive use of SMFS in folding-unfolding studies of non-metalloproteins, the use of SMFS to probe metalloproteins and metal–ligand interactions is a new emerging area that has experienced significant progress over the last few years. We will

highlight these key developments and offer a future perspective.

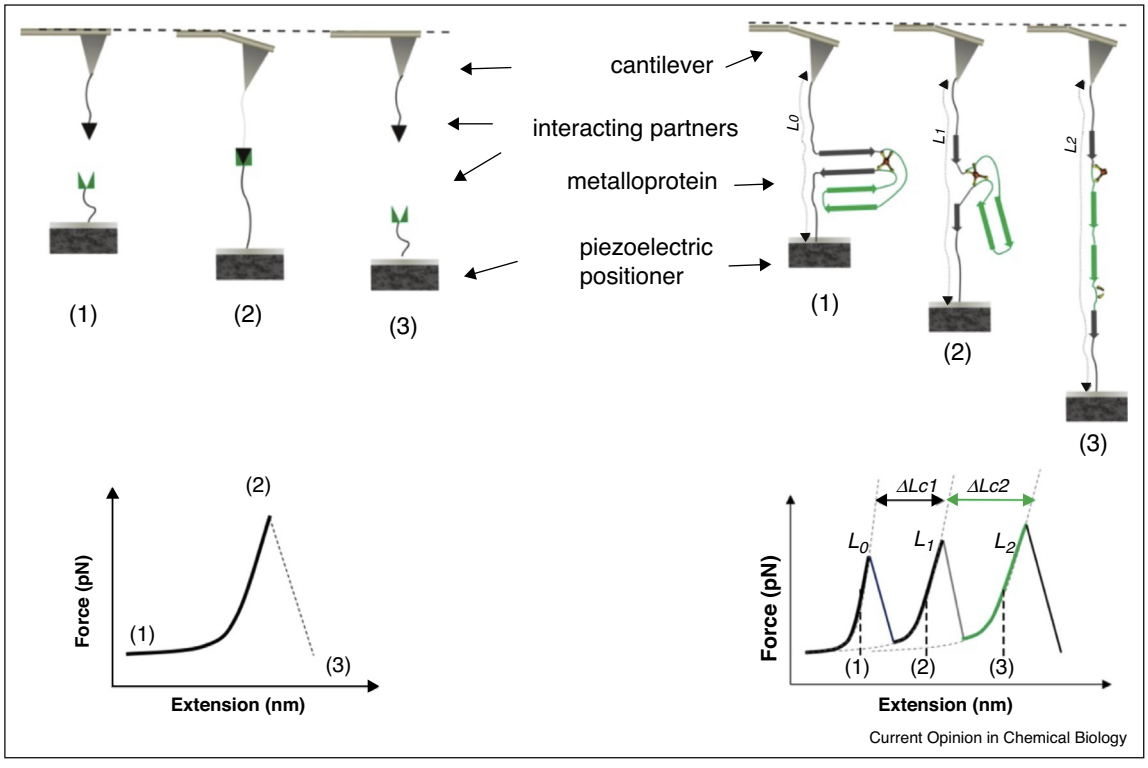
Measuring metal coordinate bond strength: from non-biological systems to metalloproteins

The development of SMFS allowed the direct measurement of the mechanical strength of metal–ligand bonds, ranging from relatively weak ones to very strong ones. Such direct measurements of chemical bond strength by single molecule AFM started from non-biological model systems. In these experiments, the interacting partners are immobilized on the AFM tip and substrate, respectively. To avoid short range non-specific interactions between the AFM tip and substrate, a polymer linker is often used as a spacer to immobilize the interacting partners [23] (Figure 1). Upon stretching, the polymer spacer extends and an entropic force develops, which directly acts upon the metal–ligand bond. When the metal–ligand bond ruptures, the rupture force can be directly recorded from the force–extension curves. And subsequent analysis will provide detailed information about the mechanical strength, free energy profile (including both free energy barrier for rupture and the width of the potential well) of the metal–ligand bond.

Au–S bond is one of the first metal–ligand bonds that have been studied using SMFS. In a pioneering SMFS study, the mechanical strength of the Au–S bond was measured using the polysaccharide amylose as a molecular linker and fingerprint for single molecule identification. It was found that the rupture force of Au–S bond is ~1.4 nN, which is consistent with the covalent nature of the Au–S bond [10•]. This result was confirmed by other studies on the mechanical strength of Au–S bond [24,25•]. The high mechanical strength of Au–S bond was also corroborated by quantum chemistry calculations [26].

Using similar strategies, a range of metal coordination bonds/interactions were measured at the single molecule

Figure 1



Principles of the AFM-based SMFS measurements of metal–ligand bond strength. Left panel: SMFS measurements of the metal–ligand bond strength. The interacting partners are immobilized onto the AFM tip and substrate via a polymer spacer. Stretching the metal–ligand bond leads to the stretching of the polymer spacer and the metal–ligand bond strength can be determined from the rupture force of the force–extension curve. Right panel: Schematics of the SMFS measurements of the mechanical strength of metal–ligand bonds in a metalloprotein and the resultant force–extension curve. *L* represents the contour length of the protein and can be measured by fitting the force–extension curve to the Worm-like chain model of polymer elasticity (dotted line). Stretching a metalloprotein containing a mechanically stable metal center will lead to the unfolding of the protein sequence outside the metal center first, giving rise a contour length increment of ΔLc1 (ΔLc1 = $L_1 - L_0$). Further stretching will lead to the rupture of the metal center, and the elongation of the polypeptide sequence sequestered by the metal center, giving rise to a contour length increment of ΔLc2 (ΔLc2 = $L_2 - L_1$). ΔLc2, which can be calculated from the number of amino acid residues sequestered by the metal center, provides an unambiguous fingerprint for identifying the metal–ligand rupture event.