2

Characterization of Protein–Protein Interactions Using Atomic Force Microscopy

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2.1. INTRODUCTION

Atomic force microscopy (AFM), invented in 1986, expanded the application of scanning tunneling microscopy to nonconductive, soft, and live biological samples (Binnig et al., 1986; Hansma et al., 1988; Marti et al., 1988; Drake et al., 1989). AFM has several capabilities, including characterizing topographic details of surfaces from the submolecular level to the cellular level (Radmacher et al., 1992), and monitoring the dynamic process of single molecules in physiological relevant solutions (Drake et al., 1989; Engel and Muller, 2000). More excitingly, AFM not only extends our “vision,” but also extends our ability to “touch and manipulate” during our exploration of the biological system at the molecular level. For example, AFM can be used to manipulate macromolecules (Zlatanova and Leuba, 2003; Bockelmann, 2004; Gutsmann et al., 2004), monitor the unfolding of proteins, RNA, and protein fibers (Carrion-Vazquez et al., 1999; Fisher et al., 2000; Zhuang and Rief, 2003; Rounsevell et al., 2004), and measure the forces between interacting molecules (Chilkoti et al., 1995; Dammer et al., 1995; Ros et al., 2004). Over the past two decades, the application of AFM has advanced our knowledge in many areas of the biological sciences including DNA (Fritzsche et al., 1997; Hansma, 2001; Hansma...
et al., 2004), RNA (Lyubchenko et al., 1992; Liphardt et al., 2001; Abels et al., 2005), chromatin (Bustamante et al., 1997; Tamayo, 2003a,b; Zlatanova and Leuba, 2003; Leuba et al., 2004), proteins (Ratcliff and Erie, 2001; Stahlberg et al., 2001), lipids (Ikai and Afrin, 2003; Henderson et al., 2004), carbohydrates (Bucior and Burger, 2004), polysaccharides (Abu-Lail and Camesano, 2003), various biomolecular complexes (Lyubchenko et al., 1995; Bustamante and Rivetti, 1996; Bonin et al., 2000; Willemsen et al., 2000; Henn et al., 2001; Safinya, 2001; Janicijevic et al., 2003a), and cellular (Ohnesorge et al., 1997) and subcellular (Henderson et al., 2004; Jena, 2004) structures.

The main focus of this review is on the application of AFM imaging in air, which is the most widely used imaging mode. However, imaging in liquids, force spectroscopy imaging, and lateral force manipulation using AFM are briefly discussed. AFM imaging is a single molecule technique that can resolve individual protein–protein and DNA–protein complexes. For example, for studying DNA–protein interactions, an ensemble of DNA–protein complexes visualized by AFM can provide snapshots of the whole dynamic process. Furthermore, using AFM, the distribution of conformations within a complex population of molecules can be characterized (Bustamante and Rivetti, 1996). Meanwhile, multiple information, such as oligomeric state of proteins, protein-induced conformational changes in DNA, DNA-binding specificities, and DNA–protein binding constants (Yang et al., 2005) can be deducted simultaneously from AFM images.

This chapter focuses only on the application of AFM for investigation of protein–protein interactions free in solution and on substrates. Biological pathway events are normally implemented by protein oligomers or multiprotein assemblies rather than single proteins. If we imagine proteins as a team of workers who have jobs to do, AFM can help us understand how the players come together to bring about functions. Specifically, AFM imaging can be used to study (1) stoichiometry and protein–protein association constant (the partnership between proteins); (2) the architecture of a protein and a multiprotein complex; (3) recognition specificity of a protein complex on nucleic acids or matrix protein (the job site for a particular protein); (4) the mechanism of action of a protein, such as DNA bending or wrapping (How is the job done?); and (5) complex actions of the same or different proteins on multiple sites on DNA that result in protein filament formation, DNA looping, DNA condensation, DNA supercoiling, nucleosome remodeling, or joining of two distinct DNA molecules (How is the job done collectively?).

2.2. USE OF AFM

2.2.1. Principles of AFM

The principle of AFM varies with the different modes of AFM operation, such as contact mode, oscillating mode, and force spectroscopy mode. In the contact mode, the AFM cantilever is deflected by the sample surface. A fixed