Biochimica et Biophysica Acta xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Review

Atomic force microscopy: A multifaceted tool to study membrane proteins and their interactions with ligands ☆

Allison M. Whited, Paul S.-H. Park *

Department of Ophthalmology and Visual Sciences, Case Western Reserve University, Cleveland, OH 44106, USA

ARTICLE INFO

Article history:
Received 4 February 2013
Received in revised form 22 March 2013
Accepted 9 April 2013
Available online xxxx

Keywords:
Imaging
Force spectroscopy
Molecular interaction
Energy landscape
Protein structure

ABSTRACT

Membrane proteins are embedded in lipid bilayers and facilitate the communication between the external environment and the interior of the cell. This communication is often mediated by the binding of ligands to the membrane protein. Understanding the nature of the interaction between a ligand and a membrane protein is required to both understand the mechanism of action of these proteins and for the development of novel pharmacological drugs. The highly hydrophobic nature of membrane proteins and the requirement of a lipid bilayer for native function have hampered the structural and molecular characterizations of these proteins under physiologically relevant conditions. Atomic force microscopy offers a solution to studying membrane proteins and their interactions with ligands under physiologically relevant conditions and can provide novel insights about the nature of these critical molecular interactions that facilitate cellular communication. In this review, we provide an overview of the atomic force microscopy technique and discuss its application in the study of a variety of questions related to the interaction between a membrane protein and a ligand. This article is part of a Special Issue entitled: Structural and biophysical characterization of membrane protein–ligand binding.

Contents

Abbreviations: AFM, atomic force microscopy; DFS, dynamic single-molecule force spectroscopy; F–D, force–distance; GPCR, G protein-coupled receptor; HOPG, highly ordered pyrolytic graphite; ICAM-1, intercellular adhesion molecule-1; LFA-1, leukocyte function-associated antigen-1; LHRH, luteinizing hormone-releasing hormone; PE40, *Pseudomonas aeruginosa* exotoxin 40; SMFS, single-molecule force spectroscopy; STM, scanning tunneling microscopy

☆ This article is part of a Special Issue entitled: Structural and biophysical characterization of membrane protein–ligand binding.

* Corresponding author. Tel.: +1 216 368 2533; fax: +1 216 368 3171.
E-mail address: paul.park@case.edu (P.S.-H. Park).

1. Introduction

Membrane proteins serve many critical roles in the cell including the transmission of information from the external environment to the inside of the cell. They sense the external environment by binding ligands that act as agonists, inverse agonists, antagonists, allosteric

0005-2736/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbamem.2013.04.011

agents, or substrates. The properties of ligands are diverse and can range from ions to small molecules, peptides, or proteins. The nature of interactions between ligands and membrane proteins and the impact that ligand binding has on membrane protein structure are areas that still require more detailed insight. Atomic force microscopy (AFM) is a technique with many different applications in biology and can be used to detect details about single molecules with a spatial resolution on the nanometer scale [1–3]. This methodological platform offers several advantages over more traditional approaches and overcomes some of the barriers in the study of membrane proteins to obtain high-resolution structural and molecular information about the native system.

The study of membrane proteins often involves procedures and manipulations that move the system away from its native condition. Membrane proteins are highly hydrophobic and are natively embedded in a lipid bilayer. Their study is often not fully amenable to traditional methods used to study soluble proteins since the presence of the membrane often interferes with such methods. To circumvent these problems, membrane proteins are extracted from their native membranes using a detergent that maintains protein functionality. Biological membranes play significant roles in protein structure and function and, therefore, it may be questionable as to whether information obtained from extracted membrane proteins accurately reflects the protein under native conditions. Additionally, membrane proteins are often labeled (e.g., fluorescent tags or a staining agent) to facilitate their detection, which can also take the system away from its native condition.

AFM does not require the labeling of proteins and allows for the study of membrane proteins within the context of a lipid bilayer and in a physiological buffer [4–6]. Thus, native properties of membrane proteins can be studied since the conditions these proteins are normally exposed to can be maintained during AFM. In this review, we discuss some of the applications of AFM to study membrane proteins and their interactions with ligands under physiologically relevant conditions and present some examples that illustrate the applications discussed.

2. Atomic force microscopy

AFM was first introduced in 1986 [7]. It was created to overcome the limitations of a related scanning probe microscopy method, scanning tunneling microscopy (STM), which was introduced a few years earlier [8]. Both methods were initially used to image surfaces with atomic resolution. In the case of STM, atomic resolution of surfaces is obtained by monitoring a tunneling current between a sharp probe and a sample surface. Thus, only conductive materials can be investigated. In contrast, atomic resolution of samples is attained in AFM by monitoring small forces applied over a surface using a sharp probe mounted on a flexible cantilever, which acts as a spring and, therefore, the method can be applied to unprocessed biological material. AFM was applied to biological samples in an aqueous environment shortly after its introduction [9,10]. Since the introduction of AFM, this multifunctional technique has found numerous applications in biology and has opened the door for unique inquiries into the structure and function of biomolecules [11,12].

The basic components of an atomic force microscope include a piezoelectric scanner, flexible cantilever containing a sharp probe, laser, photodiode detector, and feedback electronics (Fig. 1A). AFM is based on a simple principle whereby the movements of a flexible cantilever containing an atomically sharp probe are monitored. The movements of the flexible cantilever can be monitored by changes in laser deflection off of a reflective surface on the backside of the cantilever. A photodiode detector detects the changes in deflection of the laser. In many commercial systems, the sample sits on the piezoelectric scanner (e.g., Fig. 1A), which can move in all three dimensions by applying voltage to the piezoelectric material, while the cantilever remains in a fixed position. In other systems, the piezoelectric scanner is attached to the AFM cantilever holder to directly control the movement of the cantilever while the sample remains stationary. This basic set-up allows for both high-resolution imaging and probing of the molecular interactions of biological samples. Thus, the capabilities of AFM extend beyond typical image-based microscopy methods.

One of the most important aspects in the application of AFM to study biological materials is sample preparation. Biological samples must be prepared suitably for the specific AFM application and must be immobilized on a solid substrate (reviewed in [13,14]). The most common substrates used to immobilize membrane protein samples include mica, highly ordered pyrolytic graphite (HOPG), and glass (reviewed in [15,16]). Mica and HOPG are particularly useful for applications that require a clean atomically flat surface. Mica exposes a negatively charged surface while HOPG exposes a hydrophobic surface for samples to adsorb on. Glass substrates can be chemically treated and useful for the attachment of live cells. Table 1 lists the types of membrane protein preparations that are suitable for the different AFM applications discussed here.

3. Imaging membrane proteins and ligands by AFM

3.1. AFM imaging

Membrane proteins present several challenges in their study by high-resolution structural methods as discussed earlier. In addition

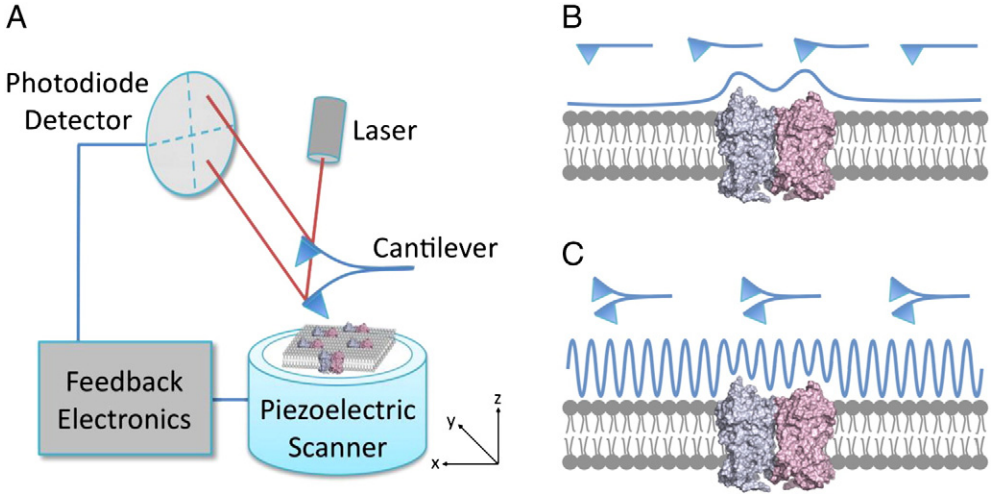

Fig. 1. AFM overview. (A) Components of an atomic force microscope. (B) Contact mode imaging. (C) Tapping mode or intermittent contact mode imaging.

Please cite this article as: A.M. Whited, P.S.-H. Park, Atomic force microscopy: A multifaceted tool to study membrane proteins and their interactions with ligands, Biochim. Biophys. Acta (2013), http://dx.doi.org/10.1016/j.bbamem.2013.04.011