Nanotechnology tools for functional proteomics analysis

Michael W. Clark, BioForce Laboratory, Inc.
Eric Henderson, BioForce Laboratory, Inc.
Will Henderson, BioForce Laboratory, Inc.
Asun Kristmundsdottir, BioForce Laboratory, Inc.
Michael Lynch, BioForce Laboratory, Inc., et al.
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BY MICHAEL W. CLARK, ERIC HENDERSON, WILL HENDERSON, ASRUN KRISTMUNDSDOTTIR, MICHAEL LYNCH, CURTIS MOSHER, AND SAJU NETTIKADAN

Now that the human genome has been fully sequenced as well as Barker’s yeast, the nematode, the fruit fly, and over 20 bacteria, the next step in this monumental research project is understanding the function and interactions of the hundreds of thousands of proteins that are encoded by those DNA sequences. Studying the proteome, which is the entire protein profile of a cell, will be the next phase in this worldwide endeavor. Functional proteomics will play an important role in understanding the action and interaction of that proteome during the functioning of a living cell.

Molecular force detection devices

Working with proteins presents unique problems not encountered when sequencing DNA. To remain in their native state and function properly, proteins must remain in environments that closely approximate their cellular milieu. Furthermore, unlike DNA, proteins are not easily amplified, so only a small quantity of protein samples will be available. A solution to these obstacles is to migrate from the conventional modes of detection to technology that works at the resolution characterized by nanotechnology. That technology is embodied in the atomic force microscope (AFM).

The design of the AFM is ideal for investigating structural characteristics and phenomena at the molecular level. The AFM can readily report topographical changes resulting from molecular complex formation and image features of single molecules. Moreover, the ability to sense extremely small forces, in the picoNewton range, provides a means to rapidly and accurately measure molecular binding affinity, molecular folding/unfolding strengths, and even electrical conductivity and magnetic characteristics of a variety of samples.

When using AFM technology, molecular force fields are imaged and imaged with nanometer resolution by bringing a specialized probe into close proximity with the sample molecule. As the probe scans across the surface, the force interactions between two surfaces and the sample result in small motions of the probe. These motions are transduced into a macroscopic signal via a sensitive laser system. A physical topographic image of that surface is then reconstructed by combining height changes recorded for each scan line. Surface features with less than a nanometer dimension can be visualized by the AFM.

The direct observation of nanometer-scale objects afforded by the AFM provides the life scientist and functional proteomics researcher with several key advantages:

- No chemical fixation of the sample
- No molecular labeling (neither radioactive, colorimetric, nor fluorescent)
- Direct, real-time visualization
- Imaging in biological buffers
- Single-molecule detection capability

The AFM provides a rapid method with nanometer resolution for the examination of a variety of molecular interactions occurring in the cell and between biological components. However, along with these benefits, biological samples present the AFM-based researcher with some special problems. Some solutions to these difficulties are described here.

Rapid and simple sample deposition

A method is needed for mounting biomolecules onto a completely clean and flat solid support surface that provides firm adhesion of the sample to the surface. Yet that adhesion, while keeping the sample in rigid placement to allow AFM probe contact, must give the biomolecule freedom to maintain a native structure and function.

For biological macromolecules, the mounting substrate must also tolerate a variety of conditions such as various ionic strengths and differing pH and temperatures. The mounting substrate needs to maintain this flexibility in deposition condition and still have a high affinity for the biological sample.

To fulfill these rigorous requirements for a sample mounting substrate, BioForce Laboratory, Inc. (Ames, IA), in collaboration with Arizona State University (Tempe, AZ), has developed AP-Mica (Figure 1). The treatment of freshly cleaved mica with aminated propyltriethoxysilane (AP) renders a newly created surface that is positively charged. This flat and clean surface is now capable of binding biomolecules in a variety of environmental conditions.

DNA or protein samples are deposited directly and firmly onto AP-Mica through a simple, one-step, self-adsorption procedure. The efficiency of DNA binding to AP-Mica is very high; 0.1 ng of DNA can be used for sample preparation. Although AP-Mica maintains a firm hold on a DNA sample, that sample can still possess relevant molecular structures (e.g., cruciform DNA). Furthermore, once prepared, the sample remains stable and uncontaminated for months with minimal controlled storage requirements.

Humidity control during AFM analysis for enhanced imaging

Biological samples function optimally in their cellular environment. That is why the AFM is an ideal tool to help the researcher regulate that environment. It does this through the AFM’s solution imaging capability using a buffer system with the proper pH and ionic conditions.

Many solution-based AFM scans can take up to 2 hr. With that long time span, the small volumes of solution utilized for the AFM (200 µL or less) can simply evaporate, drastically altering conditions. By maintaining the humidity of the imaging environment at approx. 70%, the problems resulting from evaporation of the sample buffer can be eliminated.

BioForce Laboratory has developed two solutions for monitoring and maintaining a relative humidity around the AFM probe and sample chamber for the MultiMode™ AFM (Digital Instruments-Veeco, Santa Barbara, CA).

- The HumPlug™ is a small device that fits directly into the optical head of the AFM (Figure 2). It provides benchtop convenience. The safety interlocks prevent any exposure of the operator to the intense UV.

- The TipCleaner™ is used for nanometer-scale position and manipulation of molecular specimens. The primary application is to create spatial arrays of biomolecules such as antibodies or nucleic acids.
Molecular interactions: Specialized tips for specific molecular characterizations

The interaction of the AFM probe tip with the sample can be utilized for much more than rapid, molecular structural imaging. The characteristic can be monitored depending on the composition of the AFM probe being used. By modification of an inert AFM tip with specific molecules or materials, the affinity of the sample for that specific modifying molecule can be demonstrated and recorded. The AFM probe tip is converted into an extremely sensitive, highly specific sensor that retains the sub-nanometer resolution and subatomic Newton resolution of the AFM.

The nature of the sample being examined determines the molecule used for tip derivatization. Probe tips are either directly modified or microparticles with defined biological, chemical, or material characteristics are bound to the tip. BioForce Laboratory has developed procedures for tip modification that provide maximum derivatization without loss of activity or interference with the fine operation of the AFM.

For the more routine biological and chemical AFM investigations, the company offers three major categories of modified tips: BioTips™, ChemiTip™, and MatTips™.

BioTips are biological activity probes. Typical modifications include biotin, streptavidin, Protein A, G, or A/G for antibody binding; antibodies; and Recombinant Proteins. ChemiTips are chemically active probes. Standard surface chemistries include hydrophilic (carboxyl), hydrophobic (methyl), amino active group, succinimide active group, and hydrazide active group. The active groups allow the users to apply any molecule desired that is appropriate for the active group. MatTips are probes with specific material properties. These include silica (hydrophilic), polystyrene (hydrophobic), alumina, tungsten, and various metals.

High-throughput bioaffinity binding assays

To rapidly and routinely delineate the millions of potential interactions of the newly revealed cellular proteins, any useful tool for functional proteomics must be able to process multiple samples at one time. In genomic research, the DNA microarray (a small silicon chip or glass slide with thousands to hundreds of thousands of discrete areas, each containing multiple copies of an oligonucleotide or DNA fragment) is the standard high-throughput format. These microarrays usually have the dimensions of several square centimeters. Polydimethylsiloxane (PDMS) molds that lead in the mind the small quantities of protein sample usually available. BioForce Laboratory is developing the NanoArray™ (Figure 4), a nanotechnology-scale multiples assay array format for biomolecules. To construct these nanoscale biomolecular arrays, the company developed the NanoArrayer™ (Figure 5), which automatically reads and evaluates the results from each NanoArray.

A solution for this problem is the TipCleaner™ (BioForce Laboratory). It is a simple and easy-to-operate device that uses a custom-designed mercury vapor lamp grid to produce intense UV irradiation and subsequently generate significant quantities of ozone (Figure 3). Typical cleaning periods vary from 5 to 30 min; thus, a probe can be cleaned just before an important scan.