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ЗОНДИРОВАНИЕ БИОМОЛЕКУЛЯРНЫХ ВЗАИМОДЕЙСТВИЙ

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BIOMOLECULAR INTERACTIONS PROBING

Аннотация. Данная статья посвящена методике СЗМ (сканирующей зондовой микроскопии), которая используется в исследовании биомолекулярных взаимодействий. В статье представлена информация о том, что такое СЗМ, основы СЗМ, механизмы визуализации и их применение, а также рассмотрено использование методики СЗМ в кристаллографии биомолекул. Основная цель статьи это проанализировать действие методики при изучении биомолекулярных взаимодействий.

Ключевые слова. СЗМ, наконечник, биомолекулярные взаимодействия, визуализация, растровое сканирование.

Abstract. This article is devoted to SPM (Scanning Probe Microscopy) method, which is used in the research of biomolecular interactions. The article presents information on what is SPM, the basics of SPM, imaging mechanisms and their applications, as well as using SPM method in crystallography of biomolecules. The main purpose of the article is to analyze the operation of SPM method in the study of biomolecular interactions.

Key words. SPM, tip, biomolecular interactions, imaging, raster-scanning.

Discerning and understanding structure–function relationships is often predicated on our ability to measure these properties on a variety of length scales. Fundamentally, nanotechnology and nanoscience might be arguably based on the precept that we need to understand how interactions occur at the atomic and molecular length scales if we are to truly understand how to manipulate processes and structures and ultimately control physical, chemical, electronic properties on more bulk macroscopic length scales. There is a clear need to understanding the pathways and functional hierarchy involved in the development of complex architectures from their simple building blocks. In order to study such phenomena at such a basic level, we need tools capable of performing measurements on these same length scales.

Atomic force microscopy (AFM), or more correctly, scanning probe microscop y (SPM) has come into the forefront as one of the most powerful tools for characterizi ng molecular scale phenomena and interactions.

SPM – what is it?

Scanning probe microscopy is founded on a fundamentally simple principle — by raster-scanning a sharp tip over a surface, and monitoring tip–
sample interactions, which can range in scope from repulsive to attractive forces to lo cal variations in temperature and viscoelasticity, it is possible to generate realspace i mages of surfaces with near-
molecular scale (and in some cases, atomic scale) resolution. One can reasonably des cribe these images as isosurfaces of a parameter as a function of (x, y, z) space.

SPM has become a very well-accepted technique for characterizing surfaces and interfacial processes with nanometer-scale resolution and precision. Emerging from efforts in the semi-conductor and physics fields, SPM has perhaps made its greatest impact in the biological sciences and the fields of soft materials.

SPM Basics

As you raster-scan a sharp tip and a surface past each other, you monitor any number of tip–surface interactions. One can then generate a surface contour map that reflects relative differences in interaction intensity as a function of surface position. Precise control over the tip–sample separation distance through the using of piezoelectric scanners and sophisticated feedback control schemes is what provides the SPM technique with its high spatial and force resolution.

Atomic force microscopy (AFM or SPM) is predicated on mapping local variat ions in the intermolecular and interatomic forces between the tip and the sample bein g scanned. In a conventional AFM, the surface is scanned with a nominally atomicall y sharp tip, typically pyramidal in shape, which is mounted on the underside of an ext remely sensitive cantilever.

The relative motion of the tip and sample is controlled through the use of piezo electric crystal scanners. The user sets the desired applied force (or amplitude dampe ning in the case of the intermittent contact imaging techniques). Deviations from thes

e set point values are picked up as error signals on a fourquadrant position sensitive p hotodetector (PSPD), and then fed into the main computer. The error signal provided to the instrument is then used to generate a feedback signal that is used as the input to the feedback control software.

Imaging mechanisms:

1) *Contact*

During imaging, the AFM tracks gradients in interaction forces, either attractive or repulsive, between the tip and the surface. The AFM uses this force gradient to generate an iso-force surface image. In contact mode imaging, the tip–sample interaction is maintained at a specific, user defined load. It is this operating mode that arguably provides the best resolution for imaging of surfaces and structures. It also provides direct access to so-called friction force imaging where transient twisting of the cantilever during scanning can be used to develop maps of relative surface friction. In contact mode imaging, this image represents either a constant attractive, or repulsive, tip– sample force, that is chosen by the user.

2) *Noncontact*

In noncontact mode imaging, the AFM tip is actively oscillated near its resonance frequency at a distance of tens to hundreds of Angstroms away from sample surface. The resulting image represents an isosurface corresponding to regions of constant amplitude dampening. As the forces between the tip and the surface are very small, noncontact mode AFM is ideally suited for imaging softer samples such as proteins, surfactants, or membranes.

3) *Intermittent Contact*

This method, in which the tip alternates from the repulsive to the attractive regions of the tip–sample interaction curve, has become the method of choice currently for most AFM-basing imaging. Intermittent contact mode AFM image can be viewed as an iso-energy dissipation landscape. Intermittent contact imaging provides access to other imaging modes, including phase imaging, which measures the phase shift between the applied and detected tip oscillations. Phase imaging is

particularly useful for studying biological systems, including adsorbed proteins and supported lipid bilayers, even in the absence of topographic contrast.

Applications

The breadth of possible applications for scanning probe microscopy seems almost endless. SPM/AFM-based investigations have provided novel insights into the structure and function of biomolecular assemblies. SPM has made inroads in different arenas, which can be separated into several key areas imaging; force spectroscopy; and nanomechanical property measurement. We note that it would be difficult to cover all possible applications of this technique and we will restrict our focus to in situ studies of biomolecular systems.

Crystallography

In situ SPM has been used with great success to study the mechanisms associated with crystal growth, from amino acids, to zeolite crystallization, and biomineralization. For protein crystals, studies have ranged from early investigations of lysozyme, to insulin, antibodies, and recently the mechanisms of protein crystal repair. It is worth mentioning that the high spatial and temporal resolution capabilities of the SPM are ideal for examining and measuring the thermodynamic parameters for these processes.

Protein Aggregation and FibrilFormation

In a related context, the self-assembly of proteins into fibrillar motifs has been an area of active research for many years, owing in large part to the putative links to diseases such as Alzheimer's, Huntingtin's, and even diabetes in the context of in vitro insulin fibril formation. In situ studies of aggregation and fibrillogenesis by SPM have included collagen, and spider silk. The clinical implications of fibril and plaque formation and the fact that in situ SPM is perhaps the only means of acquiring real-space information on these processes and structures that clinically cannot be easily assayed has driven recent investigations of insulin amyloid polypeptide (IAPP), amylin, beta-amyloid, and synuclein.

Membrane Protein Structure and Assemblies

One area in which scanning probe microscopy has made a significant impact has been in the structural characterization of membrane dynamics and protein–membrane interactions and assembly. Supported planar lipid bilayers are particularly attractive as model cell membranes and recent work has provided very detailed insights of their local dynamics and structure, as well as the dynamics of domain formation. Recently, thermal transitions in mixed composition supported bilayers have been studied by in situ SPM where the so-called ripple phase domains were seen to form as the system entered the gel–fluid coexistence regime.

Conclusion

As can be readily seen in the survey of the SPM field, it is clearly expanding both in terms of technique and range of applications. The systems are becoming more ubiquitous and certainly more approachable by the general user; however, what is clearly important is that care must be taken in data interpretation, instrument control, and sample preparation. New innovations in integrated single molecule correlated functional imaging tools will certainly continue to drive advances in this technology.

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