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AFM In Liquid: A High Sensitivity Study On Biological Membranes

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Abstract

Frequency Modulation AFM (FM-AFM) is commonly operated in ultra-high vacuum, though its inception in liquids for biological samples is relatively new. Here, we highlight the ability of FM-AFM to perform molecular resolution imaging of biomembrane surfaces and to detect individual layers of structured water at similar membrane interfaces. These studies highlight the potential of FM-AFM for studying model membranes and lipid raft systems on the molecular scale.

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A High Sensitivity Study on Biological Membranes

Frequency Modulation AFM (FM-AFM) is commonly operated in ultra-high vacuum, though its inception in liquids for biological samples is relatively new. Here, we highlight the ability of FM-AFM to perform molecular resolution imaging of biomembrane surfaces and to detect individual layers of structured water at similar membrane interfaces. These studies highlight the potential of FM-AFM for studying model membranes and lipid raft systems on the molecular scale.

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Frequency Modulation AFM

FM-AFM is a highly sensitive, Atomic Force Microscopy (AFM) technique that is commonly used for true atomic resolution imaging in ultra-high vacuum. In contrast to the conventional static mode AFM, FM-AFM is a dynamic technique where the cantilever is used as a mechanical resonator in a self-oscillation circuit such that the cantilever vibrates at is resonance frequency with a constant oscillation amplitude. The frequency shift (Δf) of the cantilever induced by the tip-sample interaction force is detected and used for feedback to control the tip-sample separation distance. In addition, the dissipation involved in the tip-sample interaction can also be measured by monitoring the driving voltage needed to keep the oscillation amplitude constant. This approach enables a highly sensitive force feedback control that is fully quantitative, and separates out the conservative and dissipative components of the interaction force. In practice, FM-AFM has successfully been applied to various biological systems in both the imaging and force spectroscopy modes [1,2], and recently shown to yield true atomic resolution in liquid [3]. Here, we describe the application of FM-AFM to the study of artificial supported lipid membranes (SLB) and further discuss the work in the context of "lipid raft" systems. The latter describes specialized functional domains in the plasma membrane of cells [4], though they are currently subject to intense scrutiny [5].

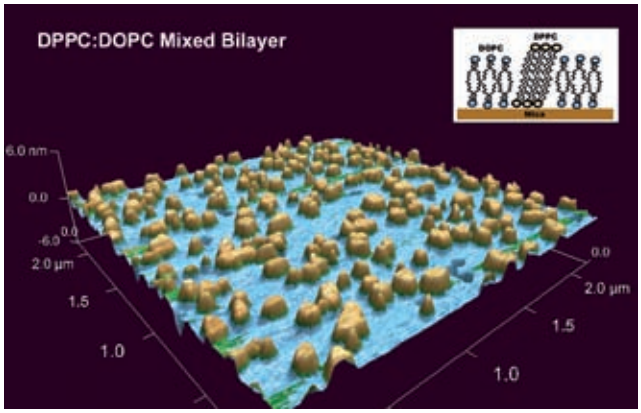

Fig. 1: AFM intermittent contact mode, 3-D height image of mixed DPPC:DOPC lipid bilayer taken in pure water at 24 °C.

AFM imaging of Multicomponent Supported Lipid Membranes

One successful approach for studying lipid rafts is to use multicomponent artificial membranes supported on solid substrates (SLB). These are known to conserve their fluidity, to some extent their overall biomimetic properties and have emerged as useful models for providing information on membranes with lipid components representing those of

lipid rafts. SLB were recognized early as excellent systems for the application of AFM, and with interest in lipid rafts growing rapidly, the study of multicomponent SLB using AFM is itself becoming a significant area of research [6]. In binary mixtures of phospholipids with different melting temperatures (Tm), the lipids can phase separate into domain structures. Higher (Tm) lipids in the gel (L_β), well-ordered state phase separate to form domains that coexist with domains enriched in lower (Tm) lipids in the disordered, fluid (L_α) state. For example, Figure 1 shows a 3-D AFM height image of an SLB consisting of two lipid components, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC). Higher regions correspond to the formation of small DPPC domains, while the lower surface (blue area) corresponds to a greater coverage of DOPC. The inset shows a schematic illustrating the difference in height between the two domain regions. At room temperature, the acyl chains of the DOPC are disordered and condensed (e.g. melted) resulting in domains with lower height. In contrast, the coexisting DPPC domains are higher due to their well-ordered and more extended chains. To specifically investigate lipid rafts, SLB are formed using a mimicking "raft mixture" of glycerphospholipid, sphingolipids and cholesterol with a molar ratio of ≈ 1:1:1. The properties of the domains/rafts that form in these SLB can then be observed in response to varying conditions, such as cholesterol concentration, temperature or the addition of membrane proteins.

Molecular Resolution Imaging of Biomembrane Surfaces

FM-AFM provides a new approach towards understanding multicomponent SLB. In particular, the ability of FM-AFM to achieve molecular resolution on membrane surfaces was recently shown in a study on 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) [7]. Figure 2 shows a 10 x 10 nm FM-AFM height image revealing true molecular resolution of a gel (L_β) phase DPPC bilayer surface. The individual headgroups (bright spots) of the lipid molecules are easily observed and hexagonally packed with an intermolecular spacing of 5.1 Å. True molecular resolution can routinely be obtained in pure water or phosphate buffered saline solution, and without the need of adjusting the salt concentration to tune the tip-sample force interaction. Thus, these results demonstrate the increased sensitivity of the FM-AFM technique, and further open the possibility of addressing important questions by visualizing SLB and lipid raft components on the molecular scale.

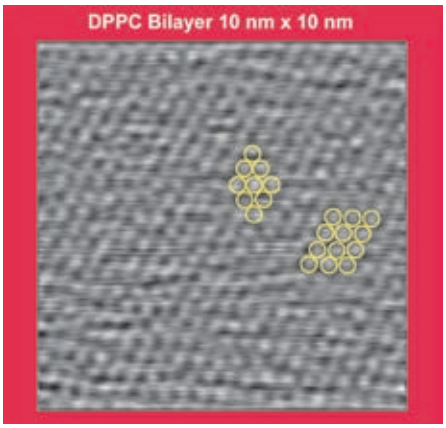

Fig. 2. Molecular resolution FM-AFM height image of gel phase DPPC bilayer taken in pure water at 24 °C.

Detection of Structured Water Adjacent to Biomembranes

Another aspect investigated using FM-AFM relates to how different lipids affect the hydration force when the bulk fluid is confined between the membrane surface and a closely approaching nanoscale object [7]. For these experiments, the AFM probe (carbon nanotube tip) approaches the membrane surface within nanometer separation and the short-range force is measured. Figure 3 shows an FM-AFM force measurement taken on a gel (L_β) phase DPPC bilayer. The curve reveals a number of clear oscillations with a spacing d approximating to the diameter of water molecule (2.8 Å) and superimposed on a background repulsive term. These oscillations reflect the molecular ordering of single water layers between the AFM probe and lipid surface, and their subsequent displacement from between the two approaching surfaces. Each rise in the oscillations is due to an increase in the finite contact stiffness of the ordered layers until at the peak maximum, the force is sufficient to displace a single water layer. In contrast, fluid (L_α) phase DOPC bilayers seriously disrupt the molecular ordering of the water molecules and predominately results in a purely monotonic profile, perhaps due to more thermally mobile headgroups and increased surface roughness which may smear out the oscillatory force. Indeed, cellular regulation of the membrane lipid composition (e.g. lipid rafts) may influence hydration in the immediate proximity of the cell surface and consequently affect the function at different regions of

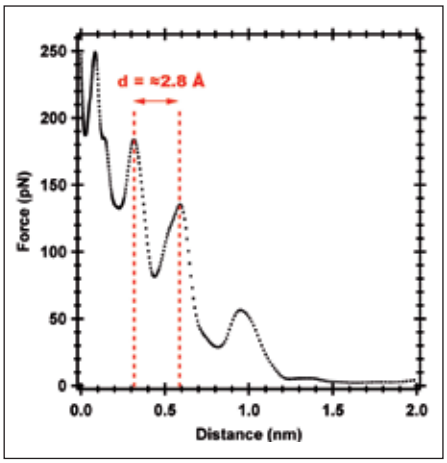

Fig. 3. FM-AFM force curve taken on gel phase DPPC bilayer in pure water at 24 °C.

the heterogeneous membrane, such as membrane diffusion or permeation.

The Lipid Raft Hypothesis

Lipid rafts are specialized functional domains in the plasma membrane that are envisaged as a mechanism for sorting lipids and aggregating associated proteins at the exclusion of others onto small (<100 nm), presumably stable-lifetime platforms [4, 8]. This new perception of a functionally relevant, laterally compartmentalized plasma membrane conceptually surpasses the original fluid mosaic model, where the membrane is viewed as an inactive fluid layer of lipid molecules with evenly dispersed, low concentrations of freely diffusing proteins. Although lipid rafts provide a constant, rational theme for explaining many cell membrane processes, their *in vivo* existence remains highly controversial due to the caveats of the evidence that defines them (i.e. detergent-resistant membranes fractions) [5]. The present conundrum is that to unequivocally prove their existence, lipid rafts must be probed in native membranes without the intervention of biochemical modification (e.g. detergent extraction). This may be problematic as lipid rafts have no defined ultrastructure and already their suggested size is down to half a dozen molecules [9], which is very close to the limit of resolution for many microscopy techniques. Thus, the current findings outlined in this paper highlight the potential of using FM-AFM to study multicomponent SLB and lipid rafts systems. Also highlighted, is the importance of considering water structure as an integral part of current cell membrane theory.

Acknowledgements

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References :

[1] Higgins M. J., *et al.,* Nanotechnology 16, 85–89 (2005)
[2] Sekiguchi H., *et al.,* Appl. Surf. Sci. 210, 54 (2003)
[3] Fukuma T., *et al.,* Appl. Phys. Lett. 87, 034101–3 (2005)
[4] Simons K. & Ikonen E. Nature 387, 569–572 (1997)
[5] Munro S. Cell 115, 377–388 (2003)
[6] Henderson R. M., *et al.,* News Physiol. Sci. 19, 39–43 (2004)
[7] Higgins M. J., *et al.,* Biophys J. Jun 23 [Epub ahead of print] (2006)
[8] Edidin M. Annu. Rev. Biophys. Struct. 32, 257–283 (2003)
[9] Hancock J. F. Nature 7, 456–462 (2006)

Further references and experimental methods are available from the authors

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