Nanoscale SPM Characterisation of Nacre Aragonite Plates and Synthetic Human Amyloid Fibres.

I. Grishin, C. Tinker, D. Allsop*, A. Robson, O.V. Kolosov

Physics Department, Lancaster University, LA1 4YB, UK, i.grishin@lancaster.ac.uk, c.tinker@lancaster.ac.uk, a.robson@lancaster.ac.uk, o.kolosov@lancaster.ac.uk
*Faculty of Health and Medicine, Lancaster University, LA1 4YQ, UK, d.allsop@lancaster.ac.uk

ABSTRACT

Atomic Foce Micoscopy is an especially useful tool in studying biological systems, because not only can they image and manipulate samples at molecular resolution, they can do so in physiological conditions – in liquid, and in air if desired as well as provide direct nanomechanical characterisation, which are essential for understanding the formation principles of these systems and the molecular mechanisms that govern their unique functionality. Nacre (mother-of-pearl), Amyloid fibres and lipid bilayer liposomes were the subject of Ultrasonic Force Microscopy (UFM) investigation.

1 INTRODUCTION

Nacre (mother-of-pearl) exhibits structural robustness despite the brittle nature of its constituents [1],[2]. This material comprises about 95% aragonite, with only a few percent of biological macromolecules acting as an interlayer, yet its work of fracture is about 3 orders of magnitude higher than monolithic ceramics, and its strength is among the highest in shell structures. Achieving a more detailed understanding of its fabrication principles developed in nature is essential for the development of new ideas in material design and synthesis. But due to this lamellar structure of Nacre, comprised of hard, brittle and soft organic components, sectioning of samples is a nontrivial task. In this study it was performed by Ion Beam Milling [3] in order to produce a highly flat section, suitable for the SPM study of the mechanical properties and morphology of the constituents and their interfaces, which has been carried out with UFM to provide true nanoscale characterisation.

The misfolding of proteins in the human body brings forth the formation of amyloids. These fibrous structures have been associated with neurodegenerative disorders like Alzheimer's disease [4]. The triggering of this misfolding is yet to be understood. A SPM study carried out on synthetic human amyloid fibres in air and under liquid brings fourth new structural details. Three different SPM techniques were used to study synthetic human amyloid fibres: Tapping mode, UFM and Under-liquid imaging with ultrasound. Various methods for sample preparation were tested, including a novel chemical deseeding technique designed to improve the quality of the synthetic human amyloid sample so it could be used to image early stage aggregates such as oligomers and protofibrils, as well volatile buffers that exclude the majority of imaging artefacts.

Liposomes consist of bilayer lipid membranes enclosing an aqueous core, which can be utilized to carry hydrophilic actives. Furthermore, liposomes with multilamellar membranes provide cargo space for lipophilic actives as well. These molecular nanostructures with well-defined particle size and shape have been the subject of eminent interest in biomedical applications (i.e., the delivery of active pharmaceuticals, imaging agents, or gene transfection) for over 4 decades. However, most liposomes are considered energetically metastable and eventually will rearrange to form planar bilayers , [5]. UFM investigation of the lipid layers' nanomechanics is presented.

Keywords: Ultrasonic Force Microscopy, Ion Beam, Nacre, Amyloid fibres, Lipid Bilayer Liposomes

2 EXPERIMENTAL DETAILS

2.1 Ion Beam Polishing.

A Leica EM TIC020 Ion Beam Cutter was used fo poducing polished sections of Nace agonite plates (Fig.1). In this method an argon ion beam is produced and directed towards the sample and shield plate to mill the unshielded part of the sample. This results in a sample cross-section produced perpendicular to the plane of the shield plate with suface roughness values around 1 nm and section width of 1-2 mm. A stereo microscope is used for sample aligning before the initiation of the procedure as well as controlling the progress of the milling.

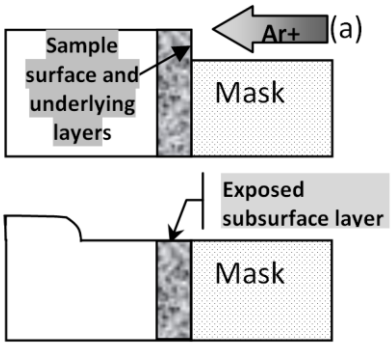


Figure 1: Schematic illustration of the principle of a Ion Beam Polishing assembly

2.2 Ultrasonic Force Microscopy

A DI Multimode AFM with additional lab test equipment (lock-in amplifier, oscloscope, arbitrary wavefom geneator) were used for the UFM mode. In this mode the sample is placed on top of a piezoelectric plate that oscillates at a frequency, that far exeeds the normal Atomic Force Microscopy cantiliver's non-contact and contact resonance frequencies. This produces the efect of a dynamicaly stiff cantiliver during contact with the sample, which in turn allows the tip to indent or elasticaly deform sample materials that are of same or higher stifness in a normal contact mode. And although there is no detectible deflection of the cantiliver on the high frequency scale (2-10 MHz) due to the high Force vs. Distance nonlinearity of a tip-surface contact a net force, or "ultrasonic" force, is detectible by the SPM at a superimposed low frequency modulation (usually of a saw tooth amplitude modulation, 1-3 KHz). This signal is interwined with the normal "linier" deflection signal and can be seperated and analysed(Fig. 2). The nonlinier deflection signal is directly related to local stiffness inhomogeneities of the sample surface and subsurface alowing for mechanical characterization of a wide range of solid state materials and structures as well as providing material composition sensitivity with no topographical variation [6],[7].

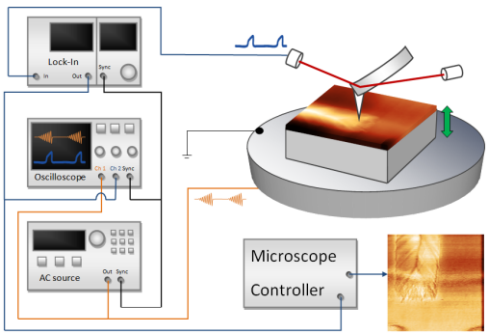
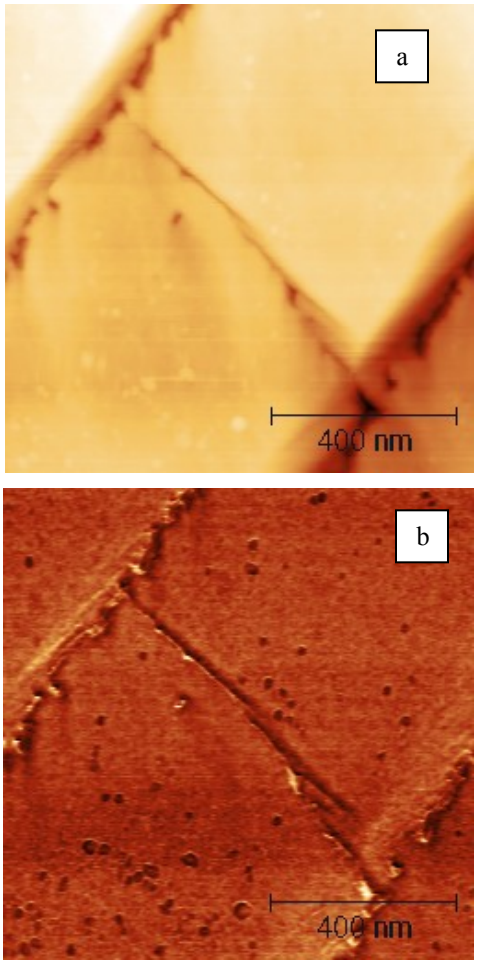


Figure 2: Schematic illustration of a UFM setup.

3 RESULTS AND DISCUSSION

3.1 Nacre

Contact mode data clearly indicates areas in the aragonite plate interfaces with nanosize mineral formations partially responsible for the excellent shearing resistance of the tablet interfaces, but as in previously reported studies [8], [9], [10] none of the features can be clearly identified as mineral bridges and not overlapping nanoasperities. The clarification is provided by Ultrasonic Force mode showing continues mechanical contrast over mineral bridges and additional interfaces between asperities and plate edges (Fig. 3 b,d).



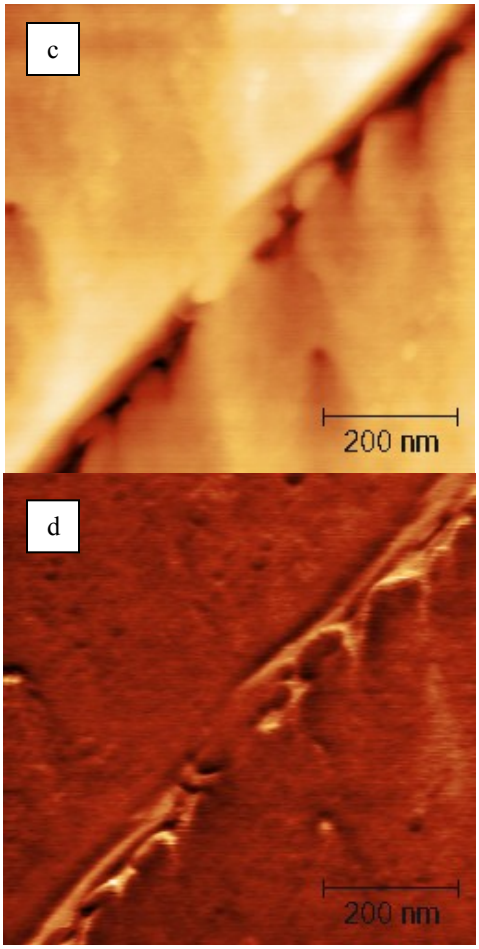


*Figure 3:*a,c)AFM topography images of Nacre argonite plates cross-section; b,d) nanomechanical elasticity images by UFM. The UFM provides a greater resolution as well as clear distinction between aragonite bridges and nanoasperities.

3.2 Amyloid Fibres

The study of deseeded samples gave the highest quality images of fibres showing clear sub-structure, as well as evidence of earlier aggregates, possibly protofibrils. Tapping mode produced accurate information about the dimensions of fibres and provided clear, sharp images that outline structural features such as helical twisting of smaller fibrils into larger fibres. UFM was found to yield a greater amount of structural information at the cost of decreased dimensional accuracy. UFM revealed that the compressibility of the fibres changes periodically as they twist, presumably when fibrils cross each other (Fig.4b). It also revealed the presence of a softer centre to several fibres, possibly indicating a helical structure assembled around a hollow core. Under-liquid imaging was performed in contact mode with and without ultrasound. Images with ultrasound produced much sharper images, allowing clearer identification of structures.

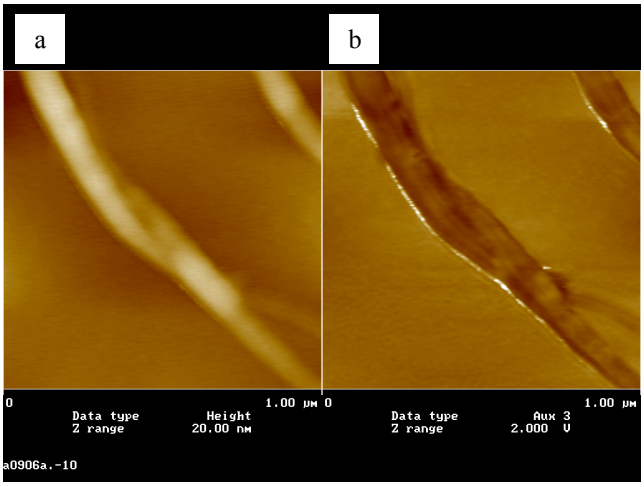
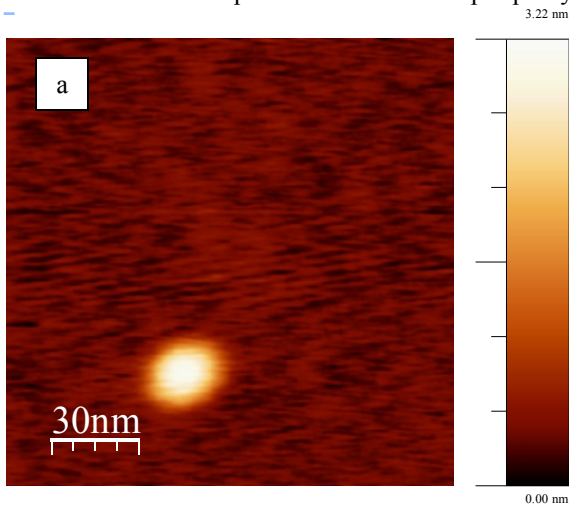


Figure 4:a) AFM contact topography image of synthetic human Amyloid fibre; b) UFM nanomechanical elasticity image. Protofibrillar structure and domains are clearly visible in the elasticity image with resolution to the nanomechanical properties on the order of 15 nm.

3.3 Liposomes

Figure 5 shows topography and nanomechanical properties of the peptide loaded lipid bilayer liposome deposited on the mica surface. The topographical profile of the liposome is smooth with UFM nanomechanical stiffness profile indicating lower stiffness at the periphery of the vesicle, that can serve as a measure of protein attached to its periphery. Due to the the periodic breaking of tip-sample contact, during a lateral scan, inherent in UFM effectively eliminates friction and hence the surface damage which would usually be caused by contact mode at higher set forces[11]. The topographical profile of the liposome is smooth and while the vesicle is significantly compressed by the AFM tip, no singularity is observed in the middle of the vesicle. At the same time, the UFM stiffness profile indicates lower stiffness at the periphery of the vesicle, that serve as a measure of protein attached to its periphery.



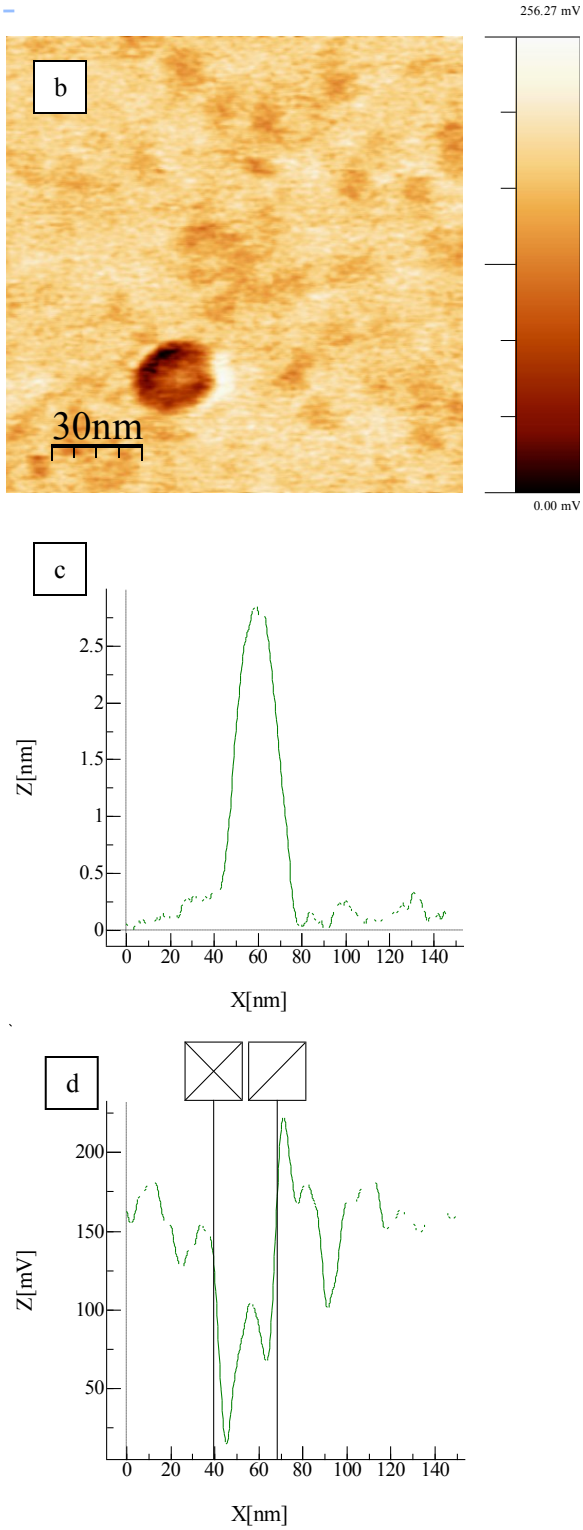


Figure 5: a) AFM contact mode topography image of liposome on mica; b) UFM elastisity image; The 1D profiles c, d) along the lines marked in a) and b) are given below the corresponding images.

4 CONCLUSIONS

Ultrasonic Force Microscopy proves to be an indispensable instrument in nanomechanical characterisation able to provide results under the handling and processing constrictions imposed by the very brittle and soft nature of a great number of scientifically and commercially relevant biological objects, such as: Nacre, Amyloid fibres and Liposomes.

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