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Using the surface spontaneous depolarization field of ferroelectrics to direct the assembly of virus particles

Working Title: Directed assembly of biological macromolecules on patterned ferroelectric surfaces

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Tobacco mosaic virus (TMV) particles have been selectively assembled at pre-determined locations on the surface of an inorganic thin film ferroelectric crystal with the composition $Pb0.3Zr0.7O3$. The domain structure and hence the distribution of surface charge on the inorganic crystal was modified using a technique that is an extension of atomic force microscopy (AFM), piezoforce microscopy (PFM). A suspension containing TMV particles was flooded across the surface of the ferroelectric before removal of the excess fluid, drying using a IR lamp and evaluation of surface distribution of the virus particles using tapping mode atomic force microscopy. The AFM images revealed that the virus particles are selectively attracted to regions of the surface that is either positively charged or nominally neutral and that regions of negative surface charge effectively repel the virus particles.

The ability of charged surfaces to attract species of opposite charge is not a new phenomenon. However, it is only in recent years that it has become possible to control

the charge distribution of a surface at micron, or smaller, length scales[1]. The ability to direct the assembly of nanoparticles or particles with functionality such as biological macromolecules, at nanometric or other small length scales, is becoming an increasing motivator for research in surface science and the broader field of nanotechnology[2]. The potential applications of directed assembly of molecules or particles that can be used in sensor and or actuator devices are varied and have the potential to have a high impact on the lives of a large number of people. It may also be possible to generate genuine 3-D nanoelectronics devices using a process of directed assembly of molecules or species on materials that have a predetermined pattern as it is possible to register to the existing pattern and then generate a new one with nanometric precision[3].

Prime of the techniques being used to achieve this surface charge modification are scanning probe microscopes[4] and high resolution scanning electron microscopes[3]. This ability to design a surface with a pattern of charge with lateral line widths as fine as 20-30nm is opening new avenues of research in fields as diverse as nano-electronics and biomimetic systems. A surface can be structured so that pre-determined regions are attractive to certain molecules or ionic species in a solution or suspension and then a useful structure can be generated from the interaction of the species and surface. The inherent ability of a ferroelectric material to sustain, for periods measured in days, a charged surface under a variety of environments ranging from aqueous solutions to vacuum allows for a wide range of interactions to be investigated. A further benefit of using ferroelectric materials is the extent to which the surface can be charged. A typical ferroelectric can develop a charge of 30ìC/cm^2 (equivalent to a surface field of 3.3 x 10$_8$

V/m), which is approximates to a fully charged surface and is just short of the field seen on ionic species[5].

In the work presented here we show that it is possible to direct the assembly of a biological macromolecule on the surface of an inorganic material using a charge pattern manually input into the substrate. The location of the virus particles on the surface of the substrate was determined by an interaction between the Stern layer associated with the ionic component of the suspension binding to the ferroelectric substrate and the double layer surrounding the virus particles. It was found that the particles were selectively assembled at the regions of the ferroelectric carrying a positive charge, this result is counter intuitive as the virus particles carry a positively charged Helm-Holz layer in the suspension medium, and is explained in terms of Stern layer interactions between the ferroelectric substrate and ionic species in the suspension. A further interesting result was the interaction between the virus particles and the nominally neutral surface regions, this indicated that these regions are positively charged and that this charging had occurred during one of the investigation stages of the experiment.

A suspension of TMV particles was produced from a sample of infected leaves. The leaves were ground up in a pestle and mortar prior to mixing with water and centrifuging, allowing the removal of the supernatant liquid containing the virus particles. This liquid was then purified to give a suspension of virus particles in a dilute solution of cellular material. The substrate of $_{Pb0.3Zr0.7O3}$ (PZT, 3 0/70) was produced using the technique of spin coating a sol-gel of precursors onto a silicon wafer coated with Ti (5nm) and Pt

(100nm). After firing to 530°C the PZT forms the ferroelectric perovskite phase that is required for the charge writing. The Ti and Pt layers are required as it is necessary to have a back electrode during the charge writing procedure[6]. Charge was written into the substrate using an atomic force microscope that has been PFM enabled. A simple pattern of four squares, two written positively and two negatively within a neutral surface was generated in the substrate. Each of the squares was programmed to be 2.5 ìm^2, however,

due to inconsistencies in the scanner (hysteresis and creep) the actual dimensions of the squares drawn varied slightly. The written charge pattern was checked using PFM, the results of which are shown in Figure 1, showing in Part a, the domain structure and Part b, the topography of the region. The feature highlighted in Part b of Figure 1 allows the referencing of further images to the charge pattern written into the substrate.

A 5ml drop of the suspension was placed over the region that had been charge written. The suspension was left for five minutes before excess fluid was removed and the surface dried using an IR lamp. Zeta potential measurements on the suspension have shown that the virus particles are positively charged in the solution at a pH of 5.5. Figure 2 shows a tapping mode AFM scan made of the charge modified region after the virus particles had been flooded over the surface. The image shows there is a higher concentration of the virus particles over the region where a positive surface charge is present than over the negative surface regions. The image also shows that there is a higher concentration of virus particles on the region of the surface that was imaged using PFM. By way of contrast to Figure 2, the normal distribution of virus particles on an unmodified/examined substrate are shown in Figure 3. The discovery that the virus particles

are attracted to a region carrying charge of the same polarity is somewhat surprising and explained due to the development of charge screening on the ferroelectric due to counter ions. In the case of the suspension the presence of anions and cations associated with the salts present allows a very stable Stern layer to form over the highly charged PZT. This layer effectively inverses the charge of the surface and explains the attraction of the particles to that area.

What is also interesting is the discovery that the virus particles are more concentrated on a region of the ferroelectric that has not been charged through domain polarisation. A larger area of the surface is shown in Figure 4, which indicates the change in the surface coverage of TMV particles over the area imaged by PFM when compared to the other regions. In this case the surface, when examined by PFM, looks to be un-changed showing a nominally neutral distribution of surface charge with individual domains ranging from 5 to 25nm. In order for the virus particles to show the higher than normal concentration there must be some interaction between the particles and the substrate. This implies that the substrate has been charged during the evaluation process.

There are two possible stages that this could occur; during the writing procedure due to stray electric fields, and the evaluation procedure when the surface charge distribution is checked. From the distribution of the charge covering exactly the region that was evaluated using PFM it seems that this is the most likely phase of charge development in the substrate. The process of evaluating the piezoelectric response of the film involves investigating the converse piezoelectric response of the film using a form of contact

AFM. During this procedure an a.c. signal of ca. 3V is applied to film as well as having the cantilever in contact with the film. The inherent bias of the film due to charge trapped at the back electrode could be responsible for a finite depth of the film becoming charged during this procedure. It would not be necessary for the film to become fully poled just for a small region at the surface to become charged in order for the virus particles to become attracted to the region that had been investigated by PFM.

The data presented in this letter shows that it is possible to use an inorganic substrate to direct the assembly of biological macro-molecules. Through the careful modification of the charge distribution in the body of the substrate it has been possible to increase the packing density of TMV particles over that expected for a non-modified surface.

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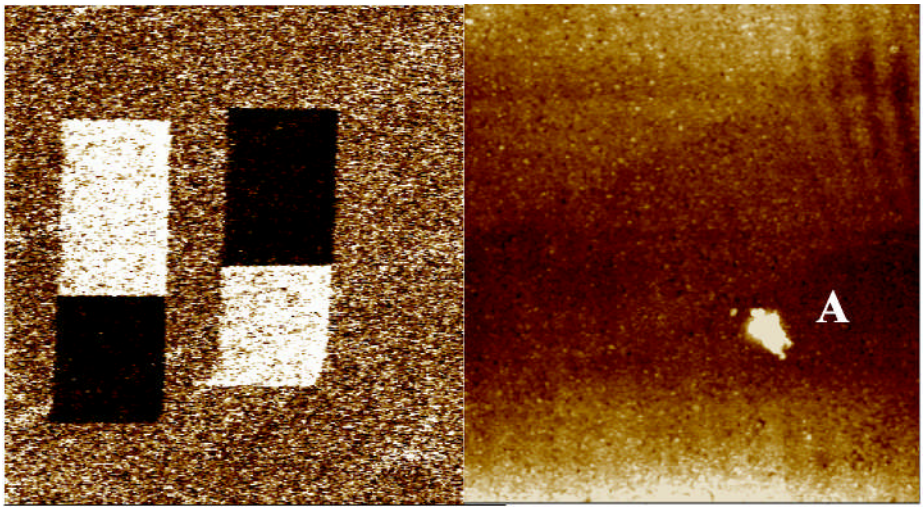
Figures



12 .5 ìm a b

Figure 1a, Charge distribution in ferroelectric as shown by PFM imaging. The white regions represent negatively charged regions, the black positively charged regions and surrounding area is nominally neutral exhibiting a domain structure ordered around 5 – 15 nm. Part b of the figure shows the contact mode topographic image of the same region. The feature marked ‘A’ allows the registering of the image with other images.

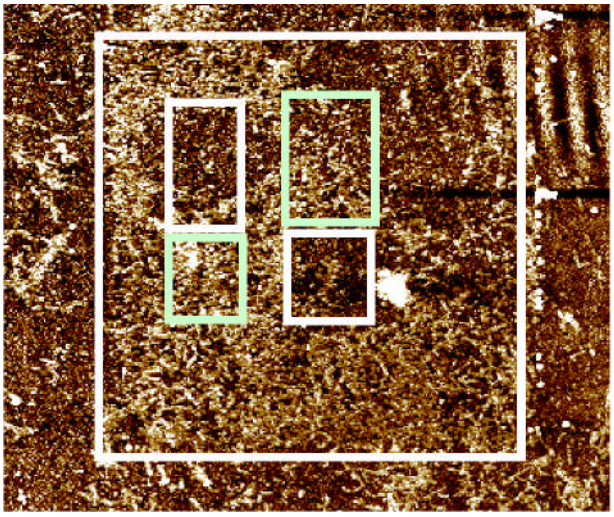


Figure 2, Distribution of virus particles on surface of ferroelectric after drying. The white boxes show the region that was investigated by PFM, highlighting the distribution of viruses in that region. A low concentration of viruses in the region that was negatively charged is clearly seen.

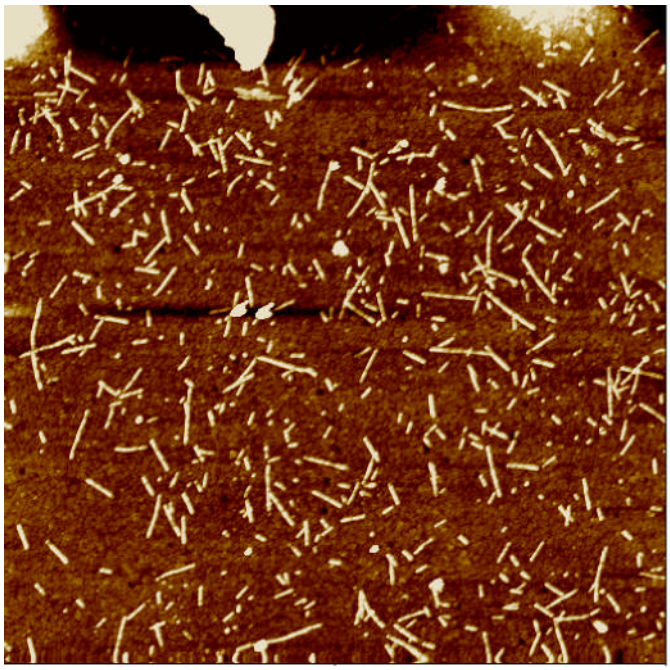


Figure 3, Normal distribution of virus particles on surface of ferroelectric material.

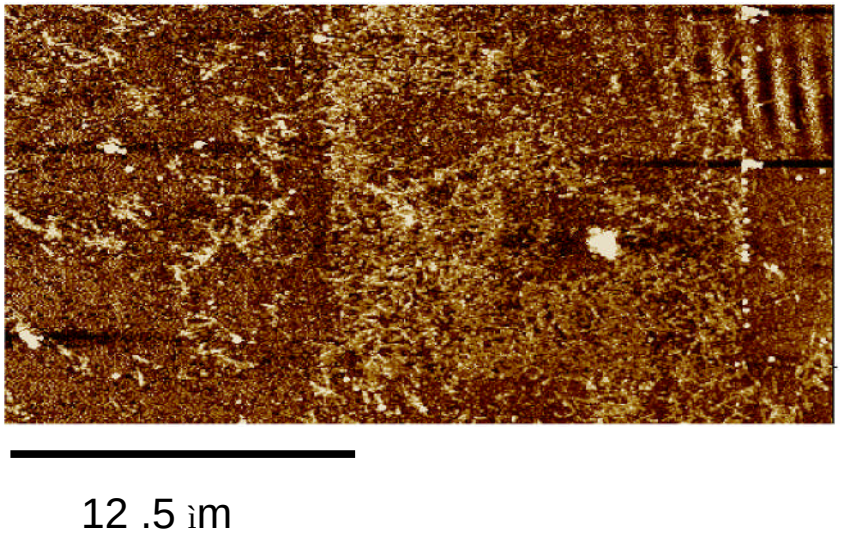


Figure 4, Large area scan including the region of the surface investigated via PFM. The increased concentration of virus particles in the region imaged via PFM is due to poling of a surface layer and the associated generation of a charged surface layer.

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