Scanning Probe Microscopy study of M13 virus on Graphite

Nathaniel Steinsultz * and Prashant Sharma **

Suffolk University, Nanoscience Center, Department of Physics, Boston, MA, USA
* nsteinsultz@suffolk.edu, ** psharma@suffolk.edu

ABSTRACT

Bacteriophage M13 belongs to a class of biomaterials that can selectively incorporate nanoparticles and self-assemble on surfaces. The structure of the virus once it is bound to inorganic substrates can only be studied by scanning probe methods. In this work, we use a combination of AFM and STM on wild-type M13 bacteriophage adsorbed on freshly cleaved graphite in ambient condition. By using non-contact atomic force microscopy (AFM) and scanning tunneling microscopy (STM) on the same sample we are able to obtain both the vertical and lateral dimensions of the virus that is bound to graphite. Using the STM we are able to resolve periodicities in the protein coat of the virus and match it to known results from X-ray diffraction analysis. We are also able to observe the location of graphite-binding motifs along the length of an M13 phage.

Keywords: M13; filamentous bacteriophage; STM; phage binding; graphite

1 INTRODUCTION

The challenge of miniaturization, or creating nanometer scale electronic devices, involves the onerous task of repeated assembly at the nanometer scale. To achieve efficient and controlled assembly of nanostructures, scientists have turned towards biological molecules [1]. In particular, there is a substantial effort towards using viruses that parasitize bacteria (bacteriophages) as building blocks in miniaturized circuits. Such an approach to nano-electronics offers a variety of applications, including nanowires and electrodes for miniaturized batteries [2][3][4]. The success of this approach derives from the properties of viruses to (a) self-assemble into different configurations, and (b) selectively bind to inorganic particles, so as to create nanoscale structures [5]. The selective binding is engineered by expressing a variety of peptide sequences (a peptide library) on the viral protein coat; panning experiments are then used to identify a peptide sequence that strongly binds to specific inorganic nanoparticles or surfaces [6].

In this paper we focus our attention on the binding of M13 bacteriophage to the surface of graphite. Conventional microscopy is unable to give details about the nature of binding and the structure of the virus that is bound to the graphite substrate. Scanning probe microscopy, including atomic force microscopy (AFM) and scanning tunneling microscopy (STM) are the usual tools for characterizing the monolayer of viruses on most substrates. Of the several scanning probe microscopes available, only the AFM has been used thus far to study the M13 bacteriophage bound to inorganic substrates [7]. The nanoscale ($\sim$ 7 nm) lateral dimension of the virus (compared to its $\sim$ 900 nm length), however, imply that force scanning microscopy tips are unable to resolve details of its structure and binding due to the usual tip-sample dilation effects. STM, on the other hand, allows for such a detailed study with good lateral resolution. Using the STM we are able to identify viruses that bind strongly to graphite. We are also able to study the structure of viruses bound to graphite surface and identify periodicities of their protein coat with the periodicities in the known structure of such filamentous bacteriophages [8][9]. The STM is well recognized as a powerful tool in studying the nature of electronic states in inorganic materials. It also has had some success in imaging biological and organic materials in ambient conditions [10][11]. To our knowledge this study is the first observation of the structure of this class of bacteriophage by scanning probe methods.

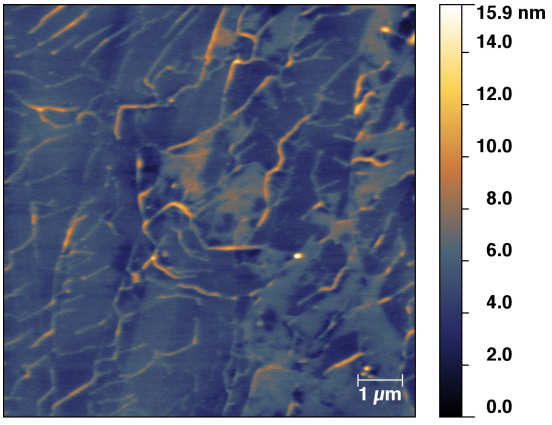

Figure 1: M13 bacteriophages imaged by AFM

2 EXPERIMENTAL SECTION

The phage display library Ph.D.-12 of bacteriophage M13 was purchased from New England Biolabs. It was diluted in ultrapure water and the concentration of M13 viral units adjusted so that there were about 10 viral units for every 5 μm^2 area on graphite surface. The graphite surfaces were prepared prior to each experiment by cleaving the highly oriented pyrolitic graphite (HOPG, grade ZYB) with an adhesive tape. The diluted solution of M13 virus was added to the surface of HOPG and allowed to incubate for an hour. The HOPG surface was then washed several times in TBS (pH 8.2) and ultrapure water to get rid of unbound viruses and salts. This method of preparation leaves behind a thin aqueous layer. The samples were imaged at relative humidity of 32% and a temperature of 22°C. The STM and AFM experiments were carried out using Agilent 5500 microscope (Agilent Technologies). AFM characterizations were operated in non-contact mode, using a typical Si cantilever with resonant frequency around 310 kHz, and both topography and phase were recorded. The STM tip was mechanically cut from Pt/Ir wires (80%/20%).

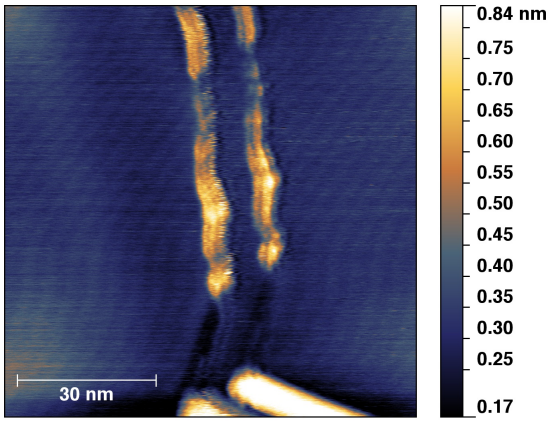

Figure 2: M13 bacteriophages imaged by STM with tunneling conditions: Tip bias $V_{\text{bias}} = -20$ mV, and current setpoint $I_{\text{set}} = 0.4$ pA. The binding strength of viruses varies along the length of a virus resulting in differing topography along the virus. Strongest binding is near the pIII end of the virus which also shows the greatest height in STM imaging.

3 RESULTS AND DISCUSSION

The bacteriophage M13 is a class of filamentous Ff bacteriophages that are about 1-2 μm long and 6-7 nm in diameter. Its cylindrical form is made up of a helical shell of protein subunits labelled pVIII, surrounding a circular DNA core. This arrangement of proteins terminates at one end into five copies of pVII and pIX protein

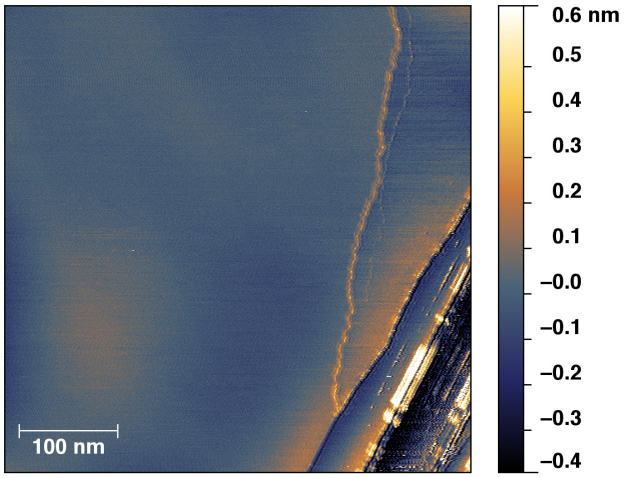

Figure 3: M13 bacteriophages imaged by STM. A doublet of bacteriophages can be seen running from the upper right side to the bottom. A fainter profile of a single bacteriophage running parallel to the viral doublet is also visible in this topographic scan. Tip bias $V_{\text{bias}} = -20$ mV, and current setpoint $I_{\text{set}} = 0.8$ pA

subunits. The infectious end of the virus terminates into five copies of pIII which are anchored to the pVIII protein through five copies of pVI protein subunit. In this study we use wild-type M13 from the PhD-12 library (a 12-mer library) on the pIII protein end of the virus. A recent study using the same library has isolated a peptide sequence on the pIII end that preferentially binds to graphite surface [12].

AFM and STM scans were used to identify a clean and relatively flat area on freshly cleaved graphite. The graphite was then incubated with the M13 virus in ultrapure water/TBS with pH 8.2. The isoelectric point of the virus is close to pH 4.0. A typical AFM scan in the non-contact mode after incubation is shown in Fig. 1 and shows the distribution of heights and lengths of the virus. It is hard to make out any difference in binding amongst the viruses based on either topography or phase and amplitude scans. The diameter of the tip used in the AFM scan is around 10 nm and it is obvious that the convolution of the tip and virus result in a greatly exaggerated lateral dimension for the virus.

For STM scans we worked at low currents (< 1 pA) to ensure that the tip-sample separation was large enough to be not too disruptive for the particles. We first obtained tunneling current I versus tip separation z data for freshly cleaved graphite. By fitting the tunneling conductance G to an exponential of the form $G = G_0 e^{-\beta z}$, where G_0 is the conductance quantum (the conductance at contact $G_0 = 77.48$ μS), we obtained a $\beta \approx 2.0$ nm^{-1}. Using this decay parameter we estimated the tip-sample separation to be around 6-7

nm in the low current tunneling range. We were unable to get stable results for all viruses that are adsorbed on graphite surface (as revealed by AFM scans), because the STM tip exerts sufficient force to knock away the weakly bound viruses. As shown in Fig. 2, strongly binding viruses are imaged by STM and the image is quite stable.

To increase the probability of finding graphite binding virus particles we doubled the density of viral units which are incubated with HOPG. STM measurements were performed after air drying the graphite surface in a relative humidity of 32%. The increased density resulted in pairing of viruses as shown in Fig. 3.

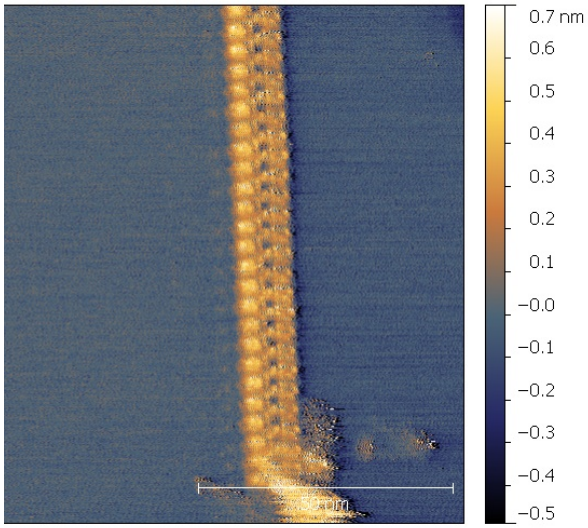

Figure 4: A doublet of M13 bacteriophages imaged by STM shows details of the pVIII protein coat. Scale bar at the bottom right is 50 nm. Tip bias $V_{\text{bias}} = -20$ mV, and current setpoint $I_{\text{set}} = 0.4$ pA

Remarkably, the doublet of viruses stay bound to the HOPG during the STM scans and retain their hydration levels, much longer than the single virus. Consequently the STM scans resolve features at a nanoscale of the doublet of viruses, shown in Fig. 4, better than those on the single virus of Fig. 2. The M13 bacteriophage's primary protein coat is made up of about 2700 copies of the pVIII protein subunits. These protein subunits are arranged in a helical shell around the virus axis as shown in Fig. 5. The end points of these protein subunits (colored blue in Fig. 5) are separated by about 3.2 nm along the axis of the virus [9]. This is the separation that we observe in the STM scans of the M13 doublet shown in Fig. 4, and in greater detail in Fig. 6.

We note that the tunneling topography across viral proteins shows no significant change when the tip bias polarity is changed from −20 mV to +20 mV. Also,

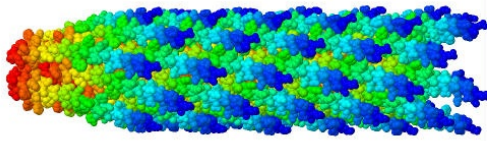

Figure 5: The pVIII protein coat of the M13 virus reconstructed from X-ray fibre diffraction and NMR data; The right end of this coat (colored red) is attached to pVI and pIII protein copies to form the infectious end of the virus (not shown). Data obtained from Protein Data Bank ID code 1ifj.

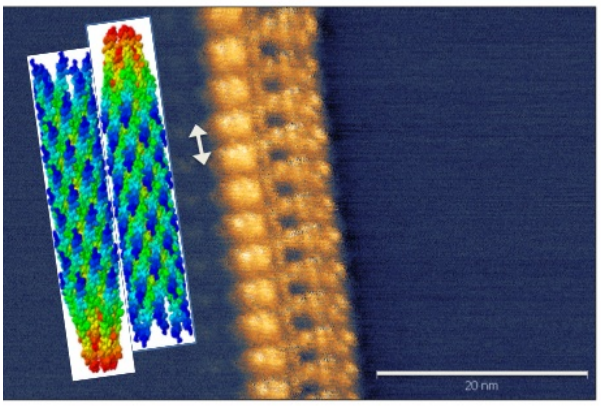

Figure 6: A doublet of M13 bacteriophages imaged by STM compared with a model of the partial structure of the viruses put together head-to-tail. The periodicities in the STM image correspond to the periodicities in the inter-virus binding. Double arrowed line represents the periodicity interval of 3.2 nm. Scale bar is 20 nm.

the viral major protein coat (pVIII) always shows up as a positive image, although the height of this image is hydration dependent ranging from $0.3 - 1.0$ nm. Further, as seen best in the M13 doublet, the tunneling topography can resolve nanoscale features of the pVIII protein coat. These facts, along with the stability of STM scan of the doublet, suggest that the tunneling current has two contributions: (a) from the graphite substrate through the bacteriophage; (b) from conductive elements of the bacteriophage protein coat bound to graphite. A more detailed study of this tunneling mechanism will be presented elsewhere.

4 CONCLUSION

To summarize, in this work we present a preliminary investigation of wild-type M13 bacteriophage bound to graphite surface using both AFM and STM. The number of isolated M13 viruses that could be reproducibly imaged by the STM was substantially less than those seen in the AFM scan for the same area of observation. This suggests that the STM tip is far more intrusive than non-contact AFM. Further, the viruses that are bound to graphite substrate show stronger binding at the pIII end than anywhere else, which is in accordance with the results of panning studies conducted in Ref. [12]. When the viruses are bound to each other as well as to graphite the STM scan can resolve the protein coat structure revealing the 32.3 $\mathring{A}$ axial repeat periodicity of the pVIII protein coat [9]. In summary, the STM study reveals the nature and strength of bacteriophage binding to conductive substrates as well as the structure of the protein coat of the virus.

REFERENCES

[1] Trevor Douglas and Mark Young. Viruses: making friends with old foes. *Science*, 312(5775):873–5, May 2006.

[2] J J Davis. Molecular bioelectronics. *Philos Transact A Math Phys Eng Sci*, 361(1813):2807–25, Dec 2003.

[3] YJ Lee, H Yi, WJ Kim, K Kang, DS Yun, MS Strano, G Ceder, and AM Belcher. Fabricating genetically engineered high-power lithium-ion batteries using multiple virus genes. *Science*, 324(5930):1051, 2009.

[4] JM Tarascon. Key challenges in future li-battery research. *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences*, 368(1923):3227, 2010.

[5] Mauricio G Mateu. Virus engineering: functionalization and stabilization. *Protein Eng Des Sel*, 24(1-2):53–63, Jan 2011.

[6] U Kriplani and B.K Kay. Selecting peptides for use in nanoscale materials using phage-displayed combinatorial peptide libraries. *Current Opinion in Biotechnology*, 16(4):470–475, 2005.

[7] SW Lee and AM Belcher. Virus-based fabrication of micro-and nanofibers using electrospinning. *Nano Lett.*, 4(3):387–390, 2004.

[8] DA Marvin, RD Hale, C Nave, and M.H Citterich. Molecular models and structural comparisons of native and mutant class i filamentous bacteriophages:: Ff (fd, f1, m13), if1 and ike. *J Mol Biol*, 235(1):260–286, 1994.

[9] DA Marvin, LC Welsh, MF Symmons, WRP Scott, and SK Straus. Molecular structure of fd (f1, m13) filamentous bacteriophage refined with respect to x-ray fibre diffraction and solid-state nmr data supports specific models of phage assembly at the bacterial membrane. *J Mol Biol*, 355(2):294–309, 2006.

[10] V Guenebaut, M Maaloum, M Bonhivers, R Wepf, K Leonard, and JKH Hoerber. Tem moire patterns explain stm images of bacteriophage t5 tails. *Ultramicroscopy*, 69(2):129–137, 1997.

[11] Mingkui Wang, Sven Bugarski, and Ulrich Stimming. Probing single flavoprotein molecules on graphite in aqueous solution with scanning tunneling microscopy. *Small*, 4(8):1110–4, Aug 2008.

[12] Y Cui, S Kim, S Jones, and L Wissler.... Chemical functionalization of graphene enabled by phage displayed peptides. *Nano letters*, Jan 2010.