Auto-orientation of G-wire DNA on mica

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Abstract

Scanning probe microscopy was used to examine the orientation of Tet1.5 quadruplex DNA polymers, a.k.a. “G-wires”, after adsorption onto freshly cleaved Phyllosilicate micas. The G-wires appear to have a preferential orientation at 60\textdegree intervals after thorough rinsing and slow drying. The angles the G-wires made with the fast scan direction of the SPM probe were measured and the frequency-angle information was quantitatively characterized by an empirical correlation coefficient. Careful measurements indicate the Tet1.5 G-wires orient along the b lattice vector of mica, the next nearest neighbor potassium vacancy. A model is proposed to explain this auto-orientation effect due to alignment of the G-wires’ phosphate backbone through magnesium tether cations. Pairs of adjacent, parallel phosphate groups of the G-wires (0.95 nm apart) appear to align with the next nearest neighbor potassium vacancy sites of mica (0.90 nm apart). This behavior is not observed in solution. The potential for using the auto-orientation phenomena in the development of high-density biomolecular nano-electronic devices is explored.

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1. Introduction

G-DNA is a polymorphic family of four-stranded structures containing guanine tetrad motifs called G-quartets [1–3]. Guanine-rich oligonucleotides that are self-complimentary, as found in many telomeric (chromosome ends) repeat sequences, form G-DNA in the presence of monovalent and/or divalent metal cations. The atomic force microscope (AFM) and the low current scanning tunneling microscope (LCSTM), high-resolution near-field three-dimensional imaging devices, were used to quantify the orientation of linear G-4 polymers on the surface of Phyllosilicate micas. These quadruplex DNA polymers, a.k.a. “G-wires” are formed by the self-assembly of G\textsubscript{4}T\textsubscript{2}G\textsubscript{4} (Tet1.5) monomers. In their seminal paper on G-wires, Marsh et al. [4] first observed that the G-wires tended to orient in the absence of any external electric field, but the phenomena was not pursued. Though the auto-orientation was suggested to be

the result of lattice alignment between the G-wires and mica, because the samples were imaged in air, the potential for drying artifacts could not be ruled out. Indeed several authors have used hydrodynamic flow [e.g. 5] and the receding meniscus methods [e.g. 6] to intentionally orient long biopolymers. Other authors have noted the propensity for orientation of DNA on mica [e.g. 7,8] and other polymers [e.g. 9,10]. These authors suggested the alignment may be due to interactions with the substrate but did not explore mechanisms to explain the orientation effect. Oriented single-wall carbon nanotubes have been demonstrated to grow on the steps of miscut sapphire crystals [11]. Our experiments indicate that step orientation does not appear to play a roll in DNA alignment. G-wires are a naturally rigid polymer [12] making observation of auto-orientation with the mica lattice easily observed, at both low and high densities (Fig. 1a and c). The nature of the orientation can be slight (Fig. 1b) to substantial (Fig. 1d) and characterized through graphical and mathematical representations of the frequency versus angle distributions. An empirical correlation coefficient is defined for use as an unbiased mechanism to determine the preferred orientation of the G-wire DNA on the mica lattice.
Factors that are observed to influence the “auto-orientation” process include length of polymer, drying, and tethering cation. The binding of duplex DNA to mica for atomic force microscopy (AFM) imaging in air is poor in the presence of monovalent cations, but enhanced by magnesium [12,13] and other multivalent cations [14]. AFM imaging of duplex DNA on bare mica in buffered media absolutely requires multivalent cations [8,15]. We propose a mechanism for auto-orientation involving a match between pairs of adjacent phosphate backbones of the quadruplex DNA with magnesium ions that replace potassium vacancies on the mica.

2. Materials and methods

G-wire DNA molecules were prepared according to the technique developed by Marsh et al. [4]. G-wires were grown from the G₄T₂G₄ monomer in a buffered solution of 50 mM NaCl, 10 mM MgCl₂, 50 mM Tris–HCl pH 7.6 at 37 °C. One microliter aliquots of concentrated G-wire solution (original Tet1.5 oligonucleotide concentration: 0.12 μg/μl) were diluted in the either of the two following “imaging” buffers containing magnesium:

1. One-Phor-all buffer (OPA): Pharmacia, 5 mM Tris–acetate pH 7.6, 50 mM sodium acetate, 5 mM magnesium acetate.
2. Magnesium ion buffer (MIB): 10 mM Tris–HCl pH 7.6, 1 mM MgCl₂.

Two microliters aliquots of the DNA/imaging buffer were directly adsorbed onto freshly cleaved muscovite mica (Ted Pella Inc.)¹ or biotite mica (Jerry’s Gems).² The samples were kept moist over exposure times ranging from seconds to weeks. No external electric fields were employed in these experiments. Samples were rinsed, gently dried with nitrogen and imaged with a Digital Instruments Nanoscope IIIa AFM or by low current scanning tunneling microscopy with a Molecular Imaging Picoscope. The orientation of the mica lattice and G-wire DNA (from same sample) were taken during a sequence of scans in which the alignment between the mica and fast scan axis of the micro-

¹ http://www.tedpella.com/.
² http://www.jerrysgemstones.com/.

Fig. 1. (a) The G-wires were imaged with a LCSTM at a relative humidity of about 60% with a bias voltages of −7 V and a tunneling current of 3.0 pA. (c) Contact AFM image of G-wire DNA networks with similar auto-orientation imaged in dry air after being incubated for 12 h in OPA buffer. Note the G-wires appear to be aligned in three preferred orientations in both images. The alignment tridents have been determined from the frequency-angle histograms (b and d). A correlation coefficient “C” (cf. Eq. (1)) indicate the G-wires align optimally at 15°, 75°, and 135° to the horizontal (LCSTM data, C = 45 ± 8%, N = 200) and 25°, 85°, and 145° to the horizontal (AFM data, C = 62 ± 8%, N = 400). The average lengths of the G-wires in (c) are 117 ± 47 nm. Vertical height scale is 10 nm from black to white in (a and c).
scope remained fixed. This assured the ability to correlate the mica lattice and G-wire orientations. A short-range scan (10 nm) was first acquired in contact mode with a fresh silicon nitride AFM tip at approximately 50% relative humidity. Higher humidity and higher contact forces seemed to improve the collection of atomic resolution images. The sample was then imaged at low humidity (<10%) and at larger scan range to collect G-wire DNA orientation data or at any humidity using Tappingmode™ AFM. LCSTM measurements were taken at 60% relative humidity, bias voltage of −7 V and a tunneling current of 3.5 pA. The lengths and orientation angles of individual G-wire segments were measured with Image SXM.3 The orientation angles were measured along the entire length of straight G-wire segments (typically around 100 nm) with the angle referenced to the fast scan direction (without electronic scanner rotation), ranging in direction from 0° to 175° and binned into 5° intervals. The correlation coefficients were determined using a Microsoft Excel macro according to Eq. (1) described below.

3. Results and discussion

The two types of buffers we used, OPA and MIB, differ in the amount of K+ (none in MIB) and Cl− (none in OPA). Both buffers appeared to work equally well indicating that neither K+ nor Cl− appears to affect the orientation results as presented through the frequency-angle histograms. Furthermore, the type of Phyllosilicate mica (two types were examined, Muscovite and Biotite) did not affect the auto-orientation results. The exposure time of the G-wire DNA in buffer to freshly cleaved mica only appeared to enhance the adhesion of longer molecules to the mica substrate without substantially affecting the auto-orientation results.

To quantitatively characterize the auto-orientation observed in Figs. 1–4 the following auto-correlation function was defined.

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3 http://www.liv.ac.uk/~sdb/ImageSXM/.
Fig. 3. (a) G-wires incubated for 10 min in OPA, rinsed, dried and imaged on APTES-treated mica in air, (b) corresponding frequency-angle histogram \( (C = 33 \pm 8\%, N = 400) \). (c) G-wires imaged in OPA on aminopropyltriethoxysilane-treated mica, (d) corresponding frequency-angle histogram \( (C = 28 \pm 8\%, N = 400) \). Note the lack of any observable preferential orientation for G-wires directly adsorbed onto APTES-treated mica. Vertical height scale is 10 nm from black to white in (a and c).

The histogram data was quantified through an empirical correlation coefficient “C” defined as the normalized sum of “binned” frequency-angle data:

\[
C = \frac{\sum \theta_{i-1} + \theta_i + \theta_{i+1} + \theta_{i+11} + \theta_{i+12} + \theta_{i+13} + \theta_{i+23} + \theta_{i+24} + \theta_{i+25}}{N}
\]

where “\( \theta_i \)” is the frequency of angles measured within a 5° interval (a.k.a. “bin”). “i” varies from 1 to 12 corresponding to angles ranging from 0° to 55° at 5° intervals. The six-fold symmetry of Phyllosilicate mica enumerates a frequency comparison at 12 corresponding sets of angles “\( \alpha \)”, “\( \alpha + 60° \)” and “\( \alpha + 120° \)”. Lastly “N” is the total number of angles measured. The frequency of angles was summed over three adjacent bins (15° interval total) to account for the ±5° error associated with angle measurements. Perfect correlation at a set of angles would correspond to a normalized correlation of 100%, i.e. all angles are located within three sets of adjacent bins, each set being 60° apart. Random correlation would correspond to a uniform distribution of frequency-angle data (8.3%/bin) of 25% (nine bins divided by 36 total bins). The largest correlation coefficient “C” out of 12 for each histogram was reported. The angle associated with the largest C was compared with the orientation of the underlying mica lattice. The error bars are estimated at worst to be about 8%, i.e. 1 bin out of 12. With this definition of error, G-wires were considered to be uncorrelated in a given histogram if the C was 33% or less, somewhat correlated if greater than 33% but less than 50% (arbitrarily chosen to be twice the random correlation value) and highly correlated if 50% or above.

According to Eq. (1), the G-wires from Fig. 1a and b would be somewhat correlated with a correlation coefficient of C = 45 ± 8%. On the other hand the G-wires in Fig. 1c and d are highly correlated with C = 62 ± 8%. Note the widths of the G-wires in Fig. 1a are about three times wider than those in Fig. 1c [16]. We believe the broadened widths in Fig. 1a are due to poor rinsing and appear wider because of the presence of excess residual salts. Though the imaging mechanisms are different (LCSTM versus Tapping AFM) we consistently find greater tendency of G-wire DNA auto-orientation from thoroughly rinsed samples. The two questions this paper attempts to address are (1) does the orientation of the G-wire DNA correlate with a particular feature of the underlying mica lattice and (2) how do we reproducibly construct these oriented G-wire networks?

The correlation coefficients were employed as an unbiased tool to determine the auto-orientation angle of the G-wire DNA. With the angle thus defined the orientation of the G-
wires with the atomic structure of the mica was then examined. To determine the atomic orientation of the potassium atoms we imaged the substrate of each sample at high-resolution and identical orientation to the fast scan axis as the low-resolution images, preferably using a short-range scanner (e.g. Digital Instruments “A” scanner). The reference direction was always the fast scan direction without electronic rotation of the piezo-electric scanner. Fig. 2a has a trimodal frequency-angle distribution \((C = 77 \pm 8\%)\). The G-wires orient at 55°, 115°, and 175°, aligning with the next nearest-neighbor potassium vacancies indicated by the white trident (Fig. 2b). The same buffer in Fig. 2a was used in fluid tapping mode imaging to determine if the auto-orientation was influenced by drying \((C = 42 \pm 8\%, \text{Fig. 2d})\). The monomodal frequency-angle distribution is reproducible for samples imaged in magnesium-based buffers. The G-wires in this image align predominantly with the nearest neighbor potassium vacancies of the freshly cleaved mica (Fig. 2e). That is the G-wires imaged in air appeared to orient in a different direction with respect to the atomic structure of mica than G-wires imaged in buffer. The mechanism of G-wire DNA orientation in buffered media on mica is the subject of further investigation.

The following control experiment was employed to rule out the possibility of auto-orientation with anything but the underlying atomic structure of mica. We treated mica with 3-aminopropyltriethoxysilane, a.k.a. “APTES” [17,18], to adsorb the G-wires by direct deposition onto an electropositive surface. We found no significant orientation when imaged in air (Fig. 3a) or in buffer (Fig. 3c). The correlation coefficients in air \((C = 33 \pm 8\%)\) and buffer \((C = 28 \pm 8\%)\) both lie in the uncorrelated region of the frequency-angle histograms (Fig. 3b and d). We therefore conclude that direct interaction between the G-wires and underlying mica substrate is essential to observe the auto-orientation effect.
Lastly we sought a way to reproducibly construct the G-wire networks seen in Fig. 1c. Following our observations that thorough rinsing reduced salt contamination and that slow drying might improve the alignment of G-wires we generated samples like those seen in Fig. 4a. The correlation coefficient for short G-wire DNA segments was $68 \pm 8\%$ (highly correlated) and for longer condensed G-wire DNA segments $C = 38 \pm 8\%$ (somewhat correlated), both from the same sample. Fig. 4b is an image of the underlying orientation of mica structure. The black trident indicates a match between the G-wire DNA orientation and the next nearest neighbor potassium vacancies at $35^\circ$, $95^\circ$ and $155^\circ$. Another reproducible result also carried out on this sample was the examination of the global effect of rinsing from a single direction. The deionized water rinse was directed from top to bottom in Fig. 4a. Note that no significant global orientation in the G-wires is observed in the vertical direction (Fig. 4c or d).

We also routinely observed that the longer segments appeared to be condensed from single strands of G-wires, identified by the white arrows. The explanation for these results now follows.

4. Discussion

The working hypothesis to explain the orientation effects has always been one based on alignment of the G-wires with the mica lattice. To be thorough a variety of other possibilities were ruled out by simple experiments. For example any gravitational affect (i.e. G-wires lying in the atomic valleys of the mica lattice) was ruled out by completing the sample preparation process with the G-wire DNA solution inverted. Indeed, the obvious electrostatic nature of the auto-orientation can be observed by the fact that even the briefest contact of G-wires to freshly cleaved mica always results in noticeable trimodal alignment after drying (e.g. Fig. 2a) with the b-lattice vector of mica. However, without drying, i.e. imaging in buffered media, a very different type of auto-orientation is observed (Fig. 2d). In buffers including magnesium-ions the G-wires appear to orient with the $a$-lattice vector of mica. This may have some important consequences of being difficult to distinguish individual molecules. Thorough rinsing and slow drying appear to be key to creating the highly oriented structures seen in Fig. 4a. Fast drying (e.g. by exposing a rinsed sample to a dry nitrogen stream) tends to result in smaller correlation coefficients for short segments (Fig. 1a). Thorough rinsing reduces the amount of excess magnesium ions that can overcome the stiffness of the G-wires and promote binding in more random orientations. We have confirmed this observation by increasing the magnesium concentration in the imaging buffer and have recorded only somewhat correlated G-wires (data not shown). We believe that slow drying of G-wire DNA segments enables the G-wires to gently adjust their positions to a lower energy state on the mica lattice. The slow evaporation observations are consistent with the kinetic trapping model of dsDNA [19] and dsRNA [20] on the surface of mica.

One of the concerns about drying the G-wires in this fashion is that the rinsing or receding meniscus might orient the G-wires due to hydrodynamic flow [5,6]. This clearly does not happen to the short G-wire DNA segments (average length $37 \pm 18$ nm, $C = 68 \pm 8\%$, Fig. 4c), but may have some impact on longer condensed segments (average length of microns, $C = 38 \pm 8\%$). Indeed, drying appears to condense multiple G-wire DNA segments (white arrows, Fig. 4a). Still, regardless of the segment lengths, the dominant angles of orientation ($35^\circ$, $95^\circ$ and $155^\circ$) were in alignment with the next-nearest potassium vacancies ($b$-lattice vector, Fig. 4b). These results are in marked contrast to the companion article by Kunstelj et al. [21], in which the guanosine monophosphate (GMP) self assembles along the $a$-lattice vector. The major building block differences between the GMP and Tet1.5 self-assembled G-wires are the pair of thymines found in Tet1.5. The thymines make for a more flexible molecule and phosphate group spacing as is evident in the differences between the images in our two papers.

The Tet1.5 G-quartets (Fig. 5b) have a phosphate backbone spacing of approximately $0.95$ nm parallel to the length of the wire (Fig. 5a and c) [22,23]. Upon drying, the G-wires appear to align themselves parallel to the next-nearest neighbor potassium vacancies spaced $0.90$ nm apart ($b$-lattice vector, Fig. 5a). The vacancies are back-filled with a divalent magnesium tethering cations. However in buffered media the alignment appears to be in the direction of the nearest neighbor vacancies, i.e. the $a$-lattice vector. Technically the phosphate backbones match the next-nearest neighbor atoms $1.04$ nm apart (Fig. 5c), as discussed by Kunstelj et al. [21]. The additional flexibility of hydrated G-wire structure [24] may be important to explain this observation. However, because of the monomodal distribution of the G-wires in solution and the nature of the imaging (tapping in fluid) we cannot rule out possible hydrodynamic impacts on the observed orientation.

The auto-orientation of G-wire DNA networks presents interesting possibilities for nanotechnology. The caged metal cations integrated into the G-wires may have some mobility [4]. In addition, the metal cations are sufficiently close to each other (about $0.3$ nm) to support electron tunneling. Lastly, the $\pi$-bonding of adjacent Guanine-quartets may overlap enough to enhance electron “hopping”. All of these mechanisms can contribute to the lateral conductivity that would make the G-wires an interesting target system for electronic biomolecular devices. In principle relevant mechanical and electronic properties of G-wires...
can be controlled by increasing or decreasing the length of the thymine linker groups, or through the substitution with adenine (e.g. G₄A₂G₄). These networks, coupled with I–V conduction experiments, are currently under investigation in our lab. Once the molecular structures of these nano-assemblies are further understood, materials with desired properties might be tailored. Because innovations in nano-circuitry rely on the development of rigid materials with predictable properties, these novel G-wire scaffolds could be used as templates for electronic nanoscale systems.

5. Summary

Dried G-wire DNA was found to be preferentially oriented along three directions 60° apart on the surface of Phyllosilicate micas in the presence of magnesium ions. Analysis of the orientations through an empirical frequency-angle correlation coefficient reveal that these directions coincide with the b-lattice vector on the basal plane of freshly cleaved mica. This orientation appears to be improved by thorough rinsing and slow drying. The ability to routinely generate G-wire networks tens of micrometers in length may be useful as a template for nanotechnology applications.

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