DNA Nano Structures Observed using Dynamic Light Scattering

TEM and AFM in Liquid Techniques

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Received 22 April 2020

Abstract: DNA nano structures have gained a lot of importance in recent times due to its potential application in the fields such as nano electronics, biosensors and programmable molecular machines. DNA nano structures are usually imaged on surfaces like mica using atomic force microscopy. In order to image DNA nano structures using atomic force microscopy, certain chemicals like magnesium acetate have to be used to fix the DNA to the substrate surface. Using chemicals to adhere the DNA to the surface may not be desirable in all cases. This is especially so if the chemical reacts with materials that are desired to be functionalized upon the DNA nano-structures. Light scattering may offer possibility to study the formation of DNA nano-structures directly from solution and the interactions between nano particles or other Biological molecules with DNA nano-structures. The study of formation of the DNA nano structures in solution has not been done before. For this research, light scattering was used to study the formation of DNA grids in solution. Light scattering offers the advantage of studying the formation of nano structures without involving chemicals to fix DNA on surfaces for imaging using AFM. Using light scattering it was observed that the formation of DNA square grids made of 4 tiles happens quickly (in about 15 minutes). The sizes of the DNA grids obtained using dynamic light scattering were found to be in agreement with those found using AFM. The formation of nano-particles and the stability of the grids were studied using TEM and Liquid mode AFM.

Keywords: DNA, Tiles, Grids, Light scattering

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1 Introduction

DNA is renowned for its function as the transmitter of genetic information, for its double helix structure and the base pairing that enables both the identification and highly selective binding of complementary DNA strands. These features, and the ability to create DNA strands with any desired sequence of bases, have led to the use of DNA rationally to design various nanostructures and even execute molecular computations [1-5]. DNA nano structures have gained a lot of importance in recent times due to its potential application in the field of nano electronics, biosensors and programmable molecular machines [5-11]. DNA has been used in the construction of patterned structures, nanomechanical devices and molecular computing systems. The formation of DNA nano structures in solution may depend upon various factors including the concentration of the individual tiles, their structures and physical and chemical conditions of the buffer. Therefore it becomes essential to study the formation of the nano structures in its early stage when the tiles are mixed in buffer solution. Techniques that involve AFM to image the DNA nano structures usually need chemicals to fix the DNA to the surfaces. This might lead to undesirable effects if one wants to study the interaction of materials (that are sensitive to the chemical fixers) with DNA nano structures. Light scattering serves as a very useful technique to probe the formation of nano structures without affecting it. Moreover unlike other imaging techniques, Light scattering offers the possibility of studying the formation of nano structures under varied conditions that include changing the buffer conditions and other physical and chemical conditions. It is also possible to study the formation of nano structures as a function of time and extract other useful details like polydispersity and particle count rates using Light scattering. Therefore Light scattering was used here to study the formation of a 4x4 DNA nano grid. The formation of nanoparticles and the stability of the grids were studied using TEM and Liquid mode AFM.

2 Experimental procedure

The tiles were prepared as outlined in [12]. Imaging by AFM was performed under 1x TAE/Mg2+ (tris acetate, 40 mM, pH 8.0), 2 mM EDTA and 12.5 mM Magnesium acetate buffer in tapping mode. Each sample to be imaged was deposited (3 μl) onto freshly cleaved mica and left for 2 min, then 25 μl of 1 x TAE/Mg2+ buffer was added to the mica and another 25 μl was placed on the AFM tip. AFM images were obtained on a digital instruments nanoscope IIIa instrument with a multimode fluid-cell head with NP-S oxide-sharpened silicon nitride tips (Vecco). To see if light scattering could be used to observe the formation of 2x2 DNA grid formed by mixing tiles T1, T2, T5 and T6 (indicated in Figure 4) DLS was used.

In this experiment with light scattering 1 μM of each tile in 1x TAE buffer was used. Quartz cuvette was used to contain the samples and the sample volume was chosen as 100 μL. The size distribution was studied using a Brookhaven Zeta Plus dynamic light scattering (DLS) system equipped with a BI-9000AT digital autocorrelator at a 659 nm wavelength. All studies were done at a 90^o scattering angle and were temperature controlled at 25^o C. The number of runs for each sample was set as 5, and the run duration was set as 1 min using the software package 9KDLSW. The correlation function was interpreted using the algorithms NNLS, CONTIN, and EXPSAM. Particle size distributions that were common with at least two of three algorithm programs were considered for data interpretation. Only those particle distributions that followed a trend were considered for data interpretation. The size distributions were also compared to the effective diameter of the particles and the particle count rate to check for correlations among the factors.

3 Results and Discussion

A 4 x 4 DNA grid has 16 tiles. Each tile is composed of a core strand, four shell strands, and four arm strands (Figure 1). The arm strands have sticky ends through which each tile gets connected. The designed distance between adjacent tile centers is 4.5 helical turns plus two DNA-helix diameters, totalling ∼19.3 nm.

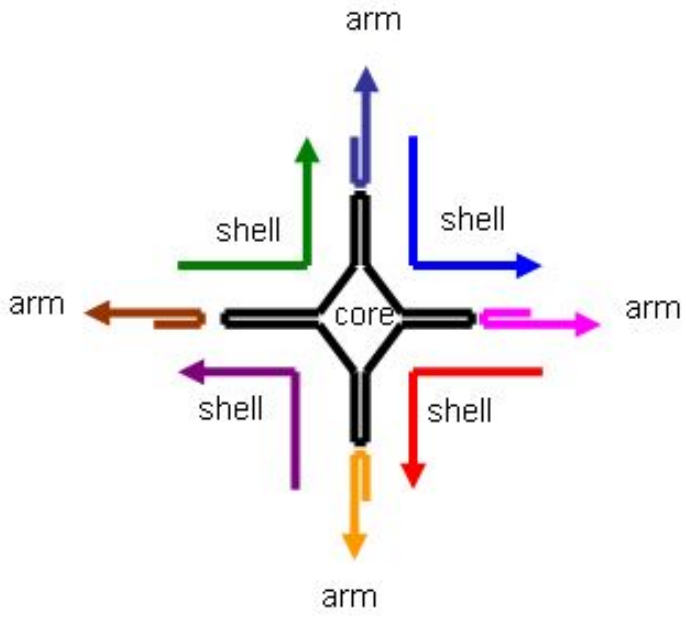

Figure 1. Tiles used to construct DNA nano grids showing arms, shells and core strands.

The images of 4 x 4 DNA grids obtained using AFM is shown in Figure 2.

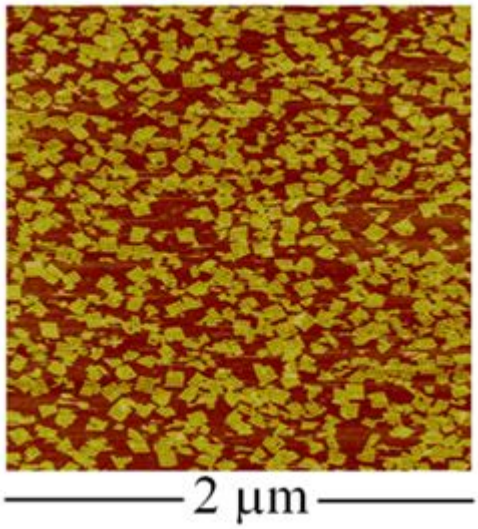

Figure 2. AFM images of 4X4 grids placed on mica surface

The distances between the centers of the tiles measure using AFM are $\sim$ 20 nm (Figures 3a and 3b).

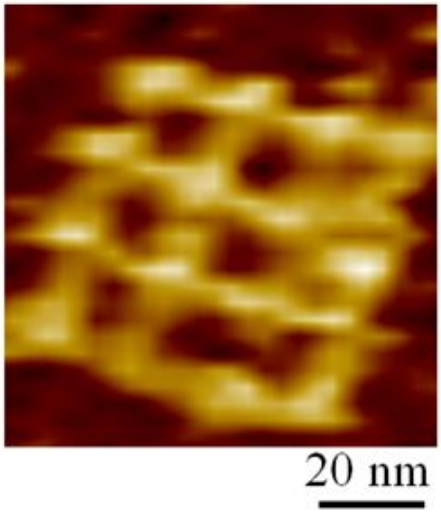

Figure 3a

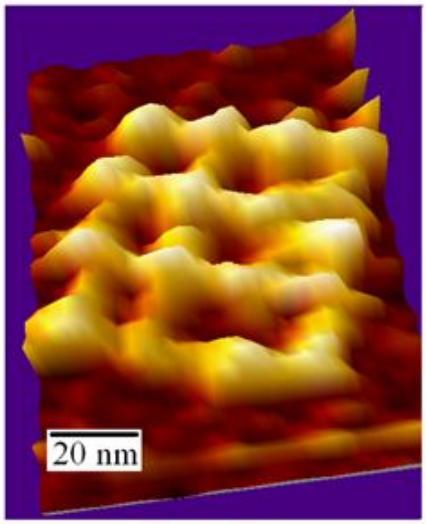

Figure 3b

Figures 3a and 3b: 2D and 3D images of 4x4 grids imaged using AFM. The distances between the centers of the tiles $\sim$ 20 nm

To see if light scattering could be used to observe the formation of 2x2 DNA grid formed by mixing tiles T1, T2, T5 and T6 (indicated in Figure 4) DLS was used.

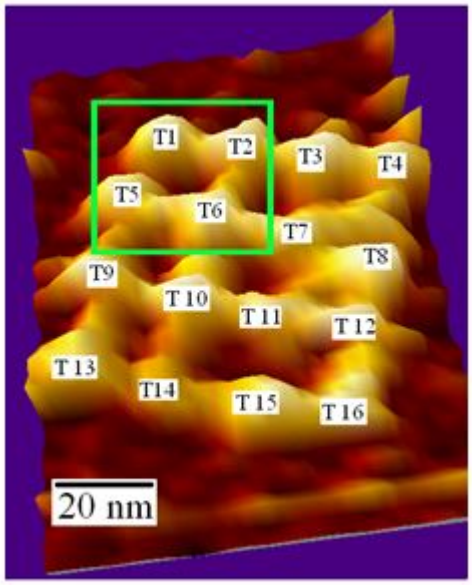

Figure 4. Tiles T1, T2, T3 and T4 form 2x2 grids when mixed together in solution.

The light scattering due to individual tiles (T1,T2,T5 and T6) and grids formed after mixing the tiles for 15, 35, 45 minutes and 18 hrs is shown in Figure 5.

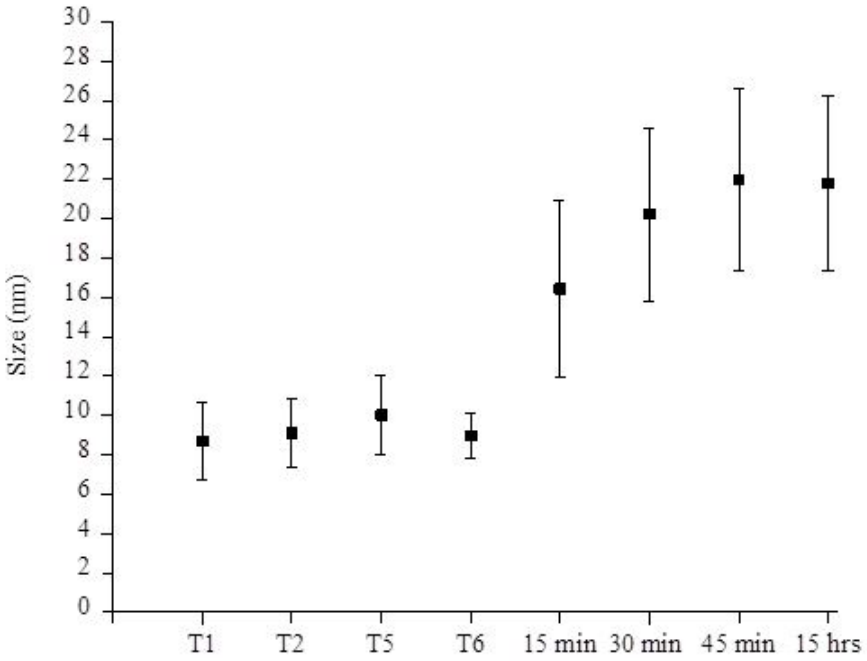

Figure 5: light scattering measurement of individual tiles (T1, T2, T5 and T6) and grids after mixing the tiles and incubating it for 15, 30, 45 minutes and 15 hrs.

The details observed using light scattering is summarized in table 1.

Table 1

Sample	Size	Particle count rate (kcps)	polydispersity	Correlation function	
				τ milli sec	C (τ)
T1	8.7 ± 2	23	0.31	8	0.014
T2	9.1 ± 1.71	19.1	0.342	8.48	0.0074
T5	10 ± 2	17	0.737	9	0.13
T6	8.98 ± 1.14	16	0.365	1	0.123
T1+T2+T5+T6 incubation time = 15 min	16.47± 4.51	61	0.362	250	0.0043
T1+T2+T5+T6 incubation time = 30 min	20.2 ± 4.3	67	0.37	377	0.0467
T1+T2+T5+T6 incubation time = 45 min	21.97± 4.65	71	0.429	63	0.0764
T1+T2+T5+T6 incubation time = 15 hrs	21.8 ± 4.42	50	0.374	66	0.081
T1+T2+T5+T6+ 1mM silver nitrate	20.4 ± 4	59	0.276	49	0.0026

It was observed that the size of the individual tiles is ~ 9 nm. When the tiles were mixed and the measurement taken, it was observed that the size measured was $\sim 20 \pm 4$ nm. This is in good agreement with the AFM measurements. The formation of the 2x2 DNA grids was complete in about 45 minutes after mixing. The sizes remained nearly the same after that even after 15 hrs. The particle count rates observed for the grids were more than that of the individual tiles. This could be because of greater scattering by DNA grids than the individual tiles as the size of the grids is greater than that of the tiles and that the scattering is proportional to the square of the diameter of the particle.

The particle count rate for the grids incubated for 15 h is lesser than that of the particle count rate at 45 min though the size measured is the same. This could be because of some grids settling to the bottom of the cuvette with time. Addition of silver nitrate solution of concentration 1mM did not change the observed sizes suggesting that the grids were stable in the presence of silver nitrate. To observe the stability of the grids in solutions favoring the formation of silver nanoparticles, UV-Vis studies, TEM studies and AFM measurements were made. Figure 6 shows the UV-Vis spectra for samples containing (i) Glutaraldehyde-silver nitrate, (ii) Glutaraldehyde-silver nitrate-Hydroquinone, (iii) Streptavidin Glutaraldehyde-silver nitrate-Hydroquinone, (iv) Bovine serum albumin (BSA)-Glutaraldehyde- silver nitrate and (v) Bovine serum albumin (BSA) - Glutaraldehyde silver nitrate-Hydroquinone. The samples were incubated for 24 h before making UV-Vis measurements.

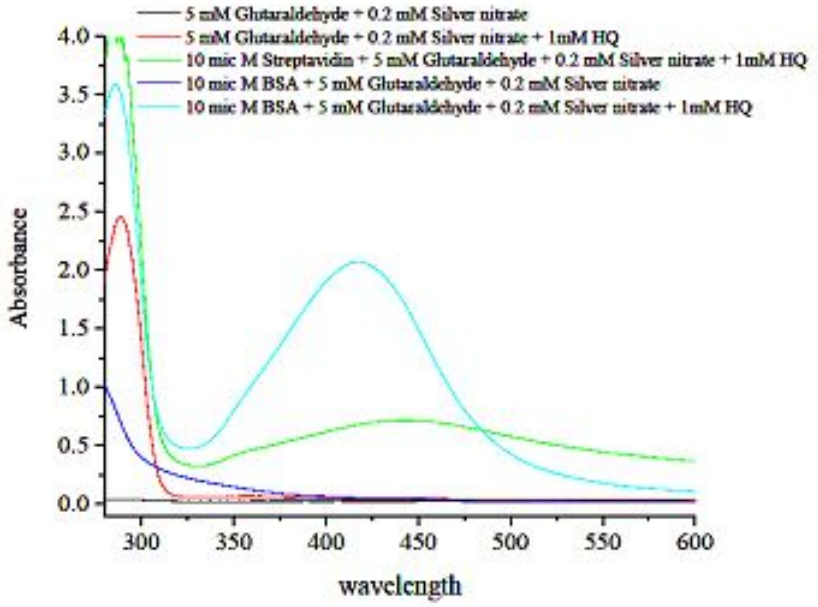

Figure 6: UV-Vis spectra

Glutaraldehyde is added to the solution to make it viscous. Solutions containing 5mM Glutaraldehyde-0.2mM Silver nitrate, 5mM Glutaraldehyde-0.2mM silver nitrate-1mM Hydroquinone and 10 mic M Bovine serum albumin (BSA)-5mM Glutaraldehyde-0.2mM silver nitrate did not show peaks between 300-600 nm indicating that nanoparticles did not form. However, 10 mic M Streptavidin-5mM Glutaraldehyde-0.2mM silver nitrate-1mM Hydroquinone and 10 mic M Bovine serum albumin (BSA)- 5mM Glutaraldehyde-0.2mM silver nitrate-1mM Hydroquinone showed prominent peak at $\sim$ 415 and 450 nm respectively indicating that proteins

such as streptavidin and BSA act as nucleation sites for the formation of stable silver nanoparticles. Figure 7 shows the TEM and SAED images of the nanoparticles formed using 10 mic M Streptavidin-5mM Glutaraldehyde-0.2mM silver nitrate-1mM Hydroquinone.

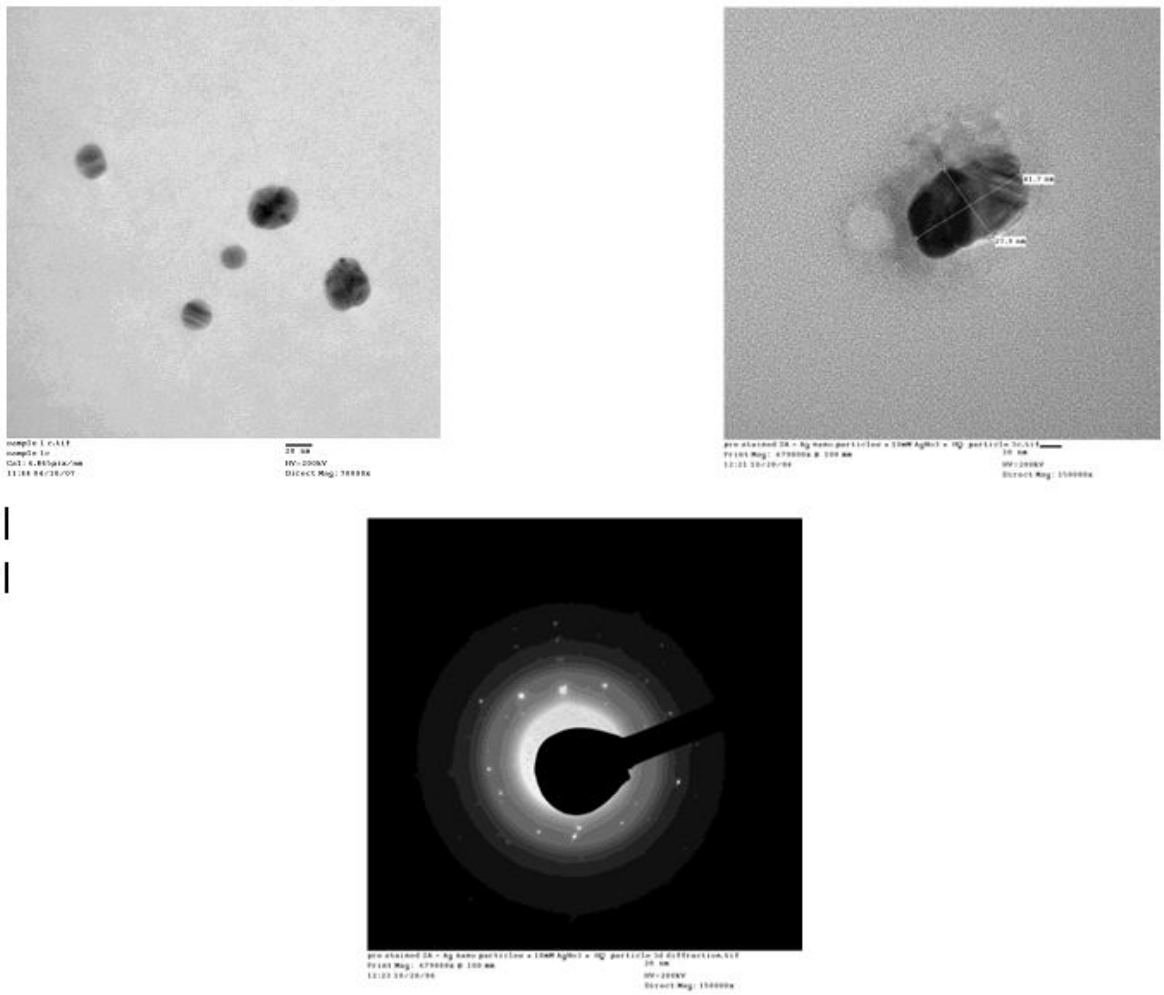

Figure 7: TEM and SAED images

The size of the nanoparticle is $\sim$ 20 - 40 nm. The Selected area diffraction (SAED) pattern shows clear spots indicating the crystalline nature of the silver nanoparticle. To study the stability of the grids the following experiment was done. To 3 nM of DNA grid 100 nM of Streptavidin was added. The ratio of Streptavidin to Glutaraldehyde was 1: 1000. After incubation for 4 hrs. 0.2 mM silver nitrate was added and incubated for another 7 hrs. Then 0.5 Mm hydroquinone was added and then it was imaged at various times. The results are shown in Figure 8.

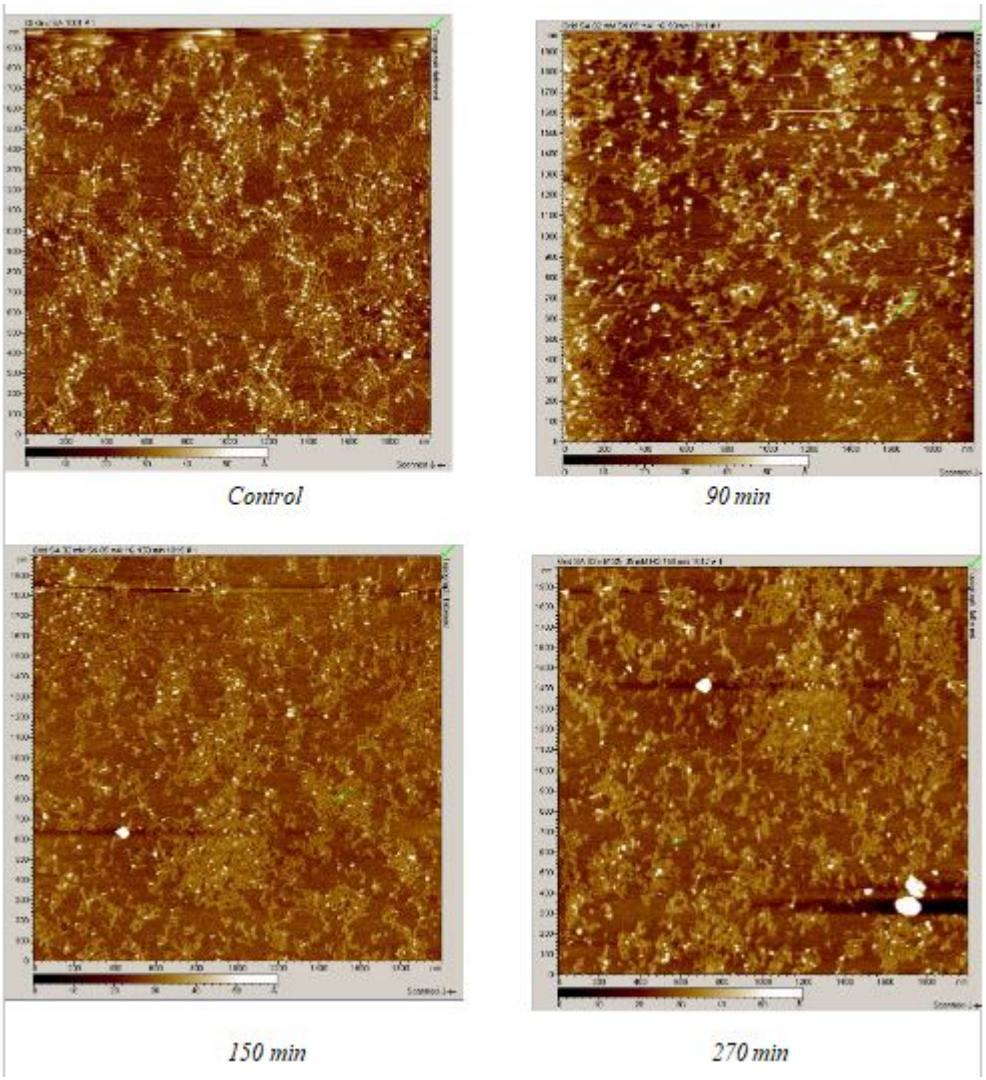

Figure 8: AFM images for DNA with streptavidin, Glutaraldehyde, silver nitrate and hydroquinone.

It is observed from Figure 8 that the streptavidin (shown as bright spots) attached to the DNA grids gradually dissociated from the DNA grids. Also, it was observed that the grids also got affected. The grids seemed to get agglomerated. However several grids could be observed to be still intact even after 270 min. These results indicate that the DNA grids are stable even for conditions favorable for formation of silver nanoparticles while the attachment of proteins (streptavidin) to the DNA grid gets affected under such conditions.

4 Conclusions

Light scattering could therefore be used to monitor the formation of DNA nano structures. These results indicate that the DNA grids are stable for conditions favorable for formation of silver nanoparticles. This study could be extended to further study the formation of DNA nano grids with tiles more than 4, having more than one squares in it (4x4, 5x 4 etc). This study could also be potentially used for studying the attachment of streptavidin molecules to DNA nanostructures having Biotin functionalized cores in it. Studying the formation of DNA nano structures along with imaging those using AFM could help in designing tiles and the order of mixing them that will give the maximum yield of grids for a given quantity of tiles. This could help reduce the cost of making DNA nanostructures by minimizing the quantity of tiles required to make them. Light scattering could possibly also be used to study the effect of temperature upon formation of DNA nano structures and also to study attachment of certain chemicals with the DNA nano structures which might be hard to image using AFM due to chemical interactions between the surfaces and materials to be functionalized on the DNA nano structures.

Acknowledgements

The author would like to thank Prof. Chris Dwyer of Department of Electrical and Computer Engineering at Duke University for providing the DNA tiles for this research. The particle size and zeta potential measurements were carried out in the laboratory of Dr. Martin Schoonen of Department of Geosciences at Stony Brook University using systems supported by the Center for Environmental Molecular Sciences (CEMS) funded by the National Science Foundation, contract number CHE0221934, contract officer Dr. Kathy Covert.

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