Study of Temperature Effects on Lambda DNA Structure Using Atomic Force Microscopy

Hasan Daw A. Mohamed[1] Mohamed Mftah Mohamed Zayed[2]
[1]Chemistry Department, Faculty of Science, Azzaytuna University, Tarhuna, Libya
[2]Medical Technology Department, high Institute of Science & technology/Awlad Ali

Hasanly274@gmail.com

تاريخ الاستلام 2024-06-28

المُلخص

تهدف هذه الدراسة إلى معرفة تأثير الحرارة على تركيب الحمض النووي DNA باستخدام مجهر القوى الذرية. تم تحضير العينات بوضع محلول من λ-DNA على رقائق صغيرة من السيلكون بعد معاملتها بمادة Chlorotrimethylsilane (Me_3SiCl). حُللت العينات المحضرة بواسطة مجهر القوى الذرية قبل وبعد تسخينها إلى 373.15، 413.15 و443.15 K لمدة 60 دقيقة. قبل التسخين، يظهر DNA على شكل جزيئات فردية ذات مظهر خيطي منتظم. أما عند تسخين العينات إلى درجة حرارة 373.15 K، يكون شكل DNA مشابه لمظهره قبل التسخين مع وجود عدد قليل من التجمعات الكروية داخل تركيب DNA والتي قد تكون نتيجة انفصال وإعادة تجمع أجزاء من DNA المحتوية على نسبة عالية من قواعد الأدنيين والثيمين. وعند تسخين العينات إلى 413.15 K يكون شكل DNA يشبه المسبحة وذلك بظهور العديد من التجمعات بمظهر شبه كروي على DNA والذي قد يرجع إلى انفصال اجزاء كبيرة من السلسلة المزدوجة. برفع درجة الحرارة إلى 443.15 K، يظهر DNA على شكل قطع صغيرة لا تتعدي اقطارها 0.5 nm، وذلك لتعرضه إلى أضرر كبيرة، حيث تتحول السلسلة المزدوجة للحمض النووي إلى اجزاء صغيرة نتيجة تكسر الروابط التي تصل النيكليوتيدات مع بعضها البعض. هذه الدراسة توضح ان الحرارة اللازمة لإحداث ضرر في تركيب DNA

عندما يكون موضوع على سطح صلب في شكل جزئيات فردية، أعلى من الحرارة اللازمة لإحداث نفس الاضرار في تركيبه عندما يكون في المحلول.

الكلمات المفتاحية: الحمض النووي DNA، التأثيرات الحرارية، مجهر القوى الذرية.

Abstract

The aim of this study was to discover the temperature influences on DNA structure using Atomic Force Microscopy (AFM) technique. For AFM measurements, a λ-DNA solution was used to align individual molecules on silicon wafers modified with chlorotrimethylsilane (Me_3SiCl). AFM imaging was conducted on DNA samples before and after being heated at 373.15, 413.15, and 443.15 K for 60 minutes. At room temperature, AFM images reveal individual structures with linear and regular morphology. At 373.15 K, DNA appears with morphologies analogous to those observed at room temperature, and structures with a small number of particles can also be seen inside the DNA structure. The globular structures might be the result of the separation and recombination of DNA segments that contain more adenine-thymine base pairs than others. With increasing temperature to 413.15 K, a number of grains were observed inside the DNA chains, which is due to the complete separation of DNA strands. At higher temperatures (443.15 K), DNA undergoes serious degradation, forming small fragments of DNA molecule. This study indicates that structural changes occur in DNA at higher temperatures when aligned on a solid substrate than when DNA is in solution. This finding may be useful in the preparation of nanostructured materials using surface-based DNA nanofabrication approach.

Key words: Atomic Force Microscopy, Deoxyribonucleic acid, Thermal degradation.

1- Introduction

Deoxyribonucleic acid (DNA) is a biomolecule that carries genetic information for all living organisms **(Niziol, et al., 2019)**. The structure of DNA is composed of two polynucleotide strands. Each nucleotide consists of a monophosphate deoxyribose sugar attached to one of four nitrogen-containing bases. These nucleotides are linked to each other via phosphodiester bonds **(Stryer, 1988)**. The two complementary single chains of DNA are attached together through hydrogen bonding between the nitrogenous bases of the DNA (adenine and thymine are paired with two H-bonds, and guanine is paired with cytosine with three H-bonds) to form a double-stranded DNA molecule **(Panigrahi, et al., 2011)**.

The DNA molecule has unique chemical and structural characteristics that make it well-suited for a wide range of applications outside of the life sciences **(Niziol et al., 2019; Zhang et al., 2014)**. This biomolecule has small size dimensions (2nm in diameter and ranging from nanometer to micron length scale) and a number of chemical binding sites, allowing it to bind a variety of chemical species through non-covalent interactions and coordinative bonding. These distinctive properties allow the DNA molecule to be used as a nanofabrication template. Indeed, a variety of nanostructured materials, including metals, polymers, and inorganic binary materials have all been prepared using DNA as a nanofabrication template **(Houlton & Watson, 2011)**.

Furthermore, DNA as a thin film can be incorporated into the structure of optical, organic, and electronic devices, resulting in a significant enhancement in their performance **(Niziol, et al., 2019; Ge et al., 2012)**. It also plays an important role in numerous diagnostic and bioanalytical applications. In such applications, elevated temperature is applied to increase performance **(Ge, et al., 2012).**

DNA molecules absorb water molecules from the air, and these molecules form two water shells around the DNA structure. The first one is known as tightly bound water, which is attached directly to DNA hydrophilic sites through hydrogen bonds. The other one is referred to as loosely bound water, which is linked to the first water shell **(Niziol, et al., 2015)**. The presence of water molecules in the DNA structure could compete with DNA binding sites for metal ion binding, thereby affecting the quality of resulting DNA templated nanomaterials. The performance of organic electronic devices can also be affected by uncontrolled ionic conduction caused by these water molecules **(Sebastiani, et al., 2014)**.

In order to enhance DNA interactions with chemical species such as metal ions and improve the performance of DNA-based thin film devices, water molecules should be removed from the DNA structure. This can be achieved by heating DNA inside an evaporating system. However, thermal energy affects the structure of the DNA molecules **(Blackburn et al., 2006)**. As the temperature increases to a certain value, the hydrogen bonds between the two DNA strands are disrupted and the helical form of the DNA backbone disappeared, resulting in the formation of two single-stranded DNA molecules **(Blackburn et al., 2006)**. Depending on the adenine-thymine and guanine-cytosine base-pair ratio, the melting of DNA in a buffer solution occurs at temperatures between 243.15 K and 263.15 K

(Blackburn et al., 2006). However, denaturation of other forms of DNA is expected to happen at higher temperatures due to a shortage of water present **(Niziol et al., 2019)**.

Moreover, the DNA molecules undergo degradation upon heating to elevated temperatures (>403.15 K). Several studies reported that the degradation process occurs when DNA molecules are heated to 493.15 K **(Jin & Grote, 2011; Wang, et al., 2001)**. This process involves the disruption of covalent bonds between DNA backbone units, resulting in the formation of smaller DNA fragments. It has been found that DNA degradation starts at 403.15 K. However, researchers have observed the presence of double-stranded DNA even after heating DNA molecules up to 473.15 K. This could be attributed to the technique used for DNA degradation and/or the short period of exposure of DNA to thermal energy **(Karni, et al., 2013; Niziol, et al., 2019)**.

In aqueous solution, the denaturation of DNA in response to temperature can be studied simply according to methods described in the literature **(Blackburn et al., 2006)**. On the other hand, many researchers still focus on the thermal stability of other forms of DNA, and a selection of protocols has already been developed **(Delport et al., 2012; Belozerova et al., 2013; Yan & Iwasaki, 2002; Ge et al., 2012)**.

One of the scanning probe microscopy (SPM) techniques that can be used to visualize structures with a resolution approaching an atomic scale is Atomic Force Microscopy (AFM). The AFM uses a nanosized tip attached to a cantilever for measuring the forces between the cantilever and the sample **(Binning, et al., 1986; Yao & Wang, 2005)**. By using this single-molecule technology, biological

samples like DNA molecules can be effectively visualized **(Pang, et al., 2015; Hansma, et al., 2004; Drew, et al., 2010; Esnault et al., 2013; Lyubchenko et al., 2014)**.

AFM has been used to investigate the structural changes of the DNA double helix induced by Dimethyl sulfoxide $(CH_3)_2SO$ **(Xu, et al., 2022)**. This scanning probe microscopy has also been used to observe the temperature impacts on the morphology of Plasmid DNA. Samples were prepared by gradually increasing the temperature of DNA solutions before AFM measurements are carried out on DNA chains aligned on a mica surface **(Yan & Iwashaki, 2002)**. In the present work, we are attempting to use AFM for investigating the effects of thermal energy on individual DNA structures isolated from solution and aligned on chemically modified silicon wafers. Several structural changes in DNA molecules induced by elevated temperatures were recorded and examined.

2- Experimental Section

2.1-Materials

Lambda DNA was obtained from New England Biolabs (UK) Ltd. Chlorotrimethylsilane (Me3SiCl) were acquired from Sigma-Aldrich and used as received. Silicon wafers (525±50 µm thickness, 1-10 Ω cm resistance, polished on one side), were acquired from Compart Technology Ltd, and used as substrate for AFM studies of DNA samples.

2.2- Surface preparation

Pieces of Si (n-100) sized (1×1 cm^2) were first treated with freshly prepared piranha solution (4:1 H_2SO_4/H_2O_2) for 45 min to clean and chemically oxidize the substrate surface. (Caution! Piranha solution is a very strong oxidant reagent and reacts aggressively with a number of chemical materials, and presents an explosion danger. Consequently). After this period of time, the silicon wafers were then washed with water and dried in an oven for approximately 10 minutes. The modification of the chemically oxidized substrate was performed through vapor deposition of a trimethylsilane (TMS): briefly, a specimen bottle containing 100 µL of chlorotrimethylsilane (Me_3SiCl) was placed in a larger specimen bottle. Then, the silicon wafer was placed on top of the inner surface. Specimen bottle (polished side facing up) and left exposed to the TMS vapor for 10 minutes at room temperature (Figure 1).

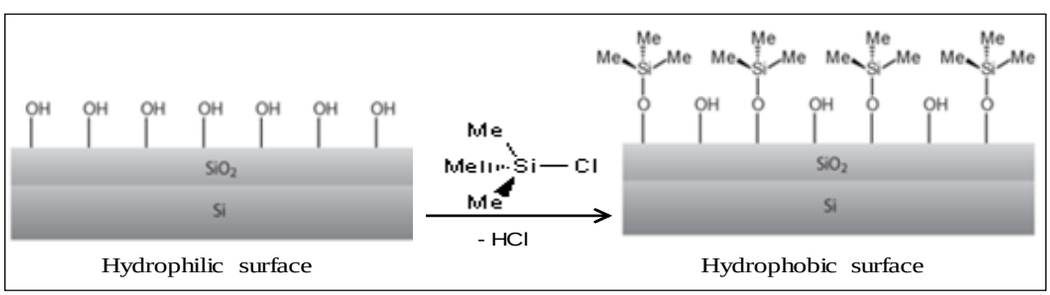

Figure 1: Representation of the modification of chemically oxidized silicon wafers with Me_3SiCl.

2.3-Alignment of λ -DNA upon TMS-modified silicon substrate

A λ-DNA solution (5 µL; 300 µg/L) was used to align the DNA on the TMS-modified substrate. This was achieved using a process based on spin-coating methods **(Mohamed *et al.*, 2012; Anabel *et al.*, 2014)**: 5 µL of the λ-DNA

solution was deposited on the TMS-modified silicon wafer while spun at 125 rpm. The substrate spinning continued for an additional 3 minutes at 250 rpm. After 3 minutes had passed, the residual DNA solution was removed from the substrate using a micropipette (figure 2). Finally, the samples were left to dry at room temperature before being examined by AFM. AFM imaging were also carried out on DNA samples after heated at different temperature degrees (373.15, 413.15, and 443.15 K) for 1 hour.

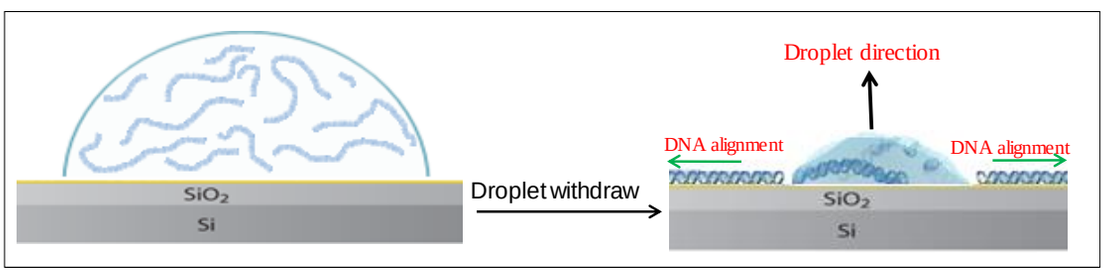

Figure 2: Illustration of the alignment of DNA on the TMS-modified silicon substrate.

2.4- Atomic force microscopy (AFM) studies

Atomic force microscopy (AFM) is one of the scanning probe microscopy (SPM) tools that can be used to visualize the structures with resolution approaching the nanoscale. A Multimode Nanoscope IIIa. in TappingMode™ operation is used to record AFM images of all DNA samples **(Rugar & Hansma, 1990; Yao, Wang, 2005)**.

3- Results and Discussion

AFM is widely recognized as a powerful tool for detecting subtle alterations in DNA structures **(Xu *et al.*, 2022)**. In the present work, λ-DNA with a precise length of 16.2 μm was used to prepare AFM samples. Sample preparation for AFM

measurements involved aligning λ-DNA molecules on silicon wafers modified with Me_3SiCl. Chemical treatments of the silicon wafers with TMS enhance the hydrophobic characteristics of the substrate surface, consequently facilitating the stretching of DNA molecules on the silicon wafers via the applied spin-coating method. This enables AFM to probe the individual structure of DNA samples **(Watson *et al*., 2013; Mohamed *et al*., 2015)**.

AFM measurements were performed on λ-DNA samples prior to and after exposure to different temperatures to study the effects of thermal energy on DNA structure. Figure 3 shows selected AFM height images of λ-DNA stretched on a TMS-treated silicon surface before and after being heated at 373.15 K, 413.15 K, and 443.15 K for 1 hour.

AFM image of λ-DNA aligned on TMS-modified Si substrate at room temperature shows frequent appearance of individual DNA structures (Figure 3a). This demonstrates that the spin-coating method is a powerful technique for stretching individual DNA structures on chemically modified substrate surfaces **(Mohamed *et al*., 2012; Anabel *et al*., 2014)**. The AFM image of DNA molecules before heating (Figure 3a) reveals reproducible smooth and regular morphology of single DNA chains. These findings align with a previous report that stated AFM imaging of λ-DNA at room temperature exhibits linear individual DNA molecules with higher-order structures **(Mohamed *et al*., 2012; Watson *et al*., 2013; Anabel *et al*., 2014; Mohamed *et al*., 2015, Hansma, et al., 1996)**. Furthermore, the heights of the measured DNA structures were found to range between 1-2 nm (cross sections in figure 3g), which is consistent with the theoretical height of a single λ-DNA structure **(Kudo & Fujihira, 2006)**.

We are now investigating the temperature effects on the λ-DNA samples. A double-stranded DNA is formed by hydrogen bonding between DNA bases, which holds two complementary single strands together. Two hydrogen bonds connect adenine to thymine, while three bonds connect guanine to cytosine. Therefore, the adenine-thymine base pairs should be easier to break when heated compared to the guanine-cytosine base pair. This point marks the discontinuity of the thermal denaturation of DNA with several thermal balance stages. The ratio of guanine-cytosine and adenine-thymine base pairs, along with the salt concentration in solution, make it a challenge to specify the temperature for DNA denaturation. Therefore, the melting temperature can be a range instead of a specific value **(Yan, *et al.*, 2002).**

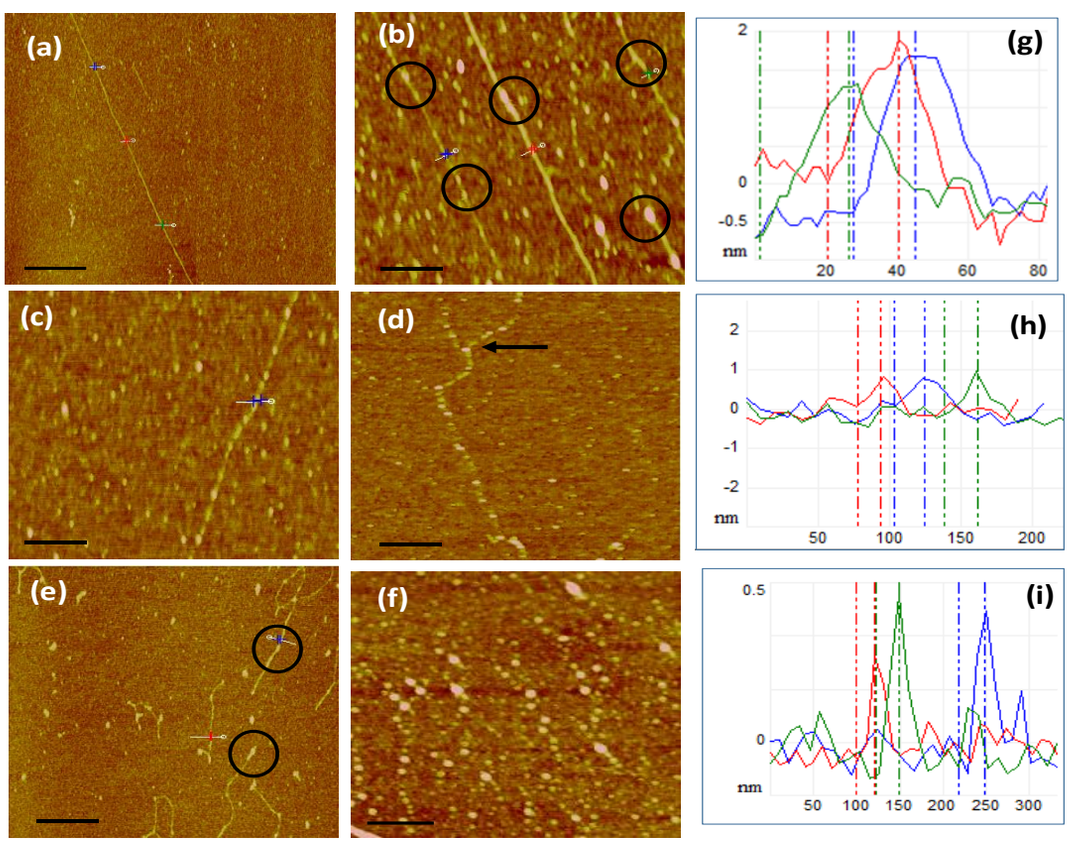

Figure3: Selected AFM height images of (a) a λ-DNA molecule aligned on TMS-modified silicon substrate at room temperature, (b) a λ-DNA molecule aligned on TMS-modified silicon substrate after being heated at 373.15 K, (c and d) a λ-DNA molecule aligned on TMS-modified silicon substrate after heated at 413.15 K, (e and f) a λ-DNA molecule aligned on TMS-modified silicon substrate after being heated at 443.15 K. (g), (h) and (i) the corresponding cross sections of (a), (c) and (e), respectively. Scale bar = 500 nm, height scale = 5 nm.

A selected AFM image of DNA after heated at 373.15 K is shown in Figure 3b. This image displays individual DNA chains aligned on the surface of silicon wafer with an average diameter of about 2 nm. These structures exhibit a smooth and uniform morphology, similar to what is observed for DNA samples at room temperature (Figure 3a). However, upon closer inspection, a small number of particles can be seen inside the structure of DNA molecules (circled area in Figure 3b). The formation of these globular structures might be a result of the separation of DNA chains that contain more adenine-thymine base pairs than others.

Such a change in DNA structure has been observed to take place at 46 °C for DNA samples prepared by heating the DNA solution before being aligned on a mica surface **(Yan, et al., 2002)**. The reason behind the difference in temperature required for the denaturation of DNA samples may be due to the absence of extra water in DNA samples that prepared by alignment of DNA molecules on solid substrate.

Two factors could contribute to the formation of these globules: (1) Once the two strands of DNA are separated, loop chains consisting of numerous base pairs become visible. These base pairs have a high chance of interacting with each other

within the chain, leading to the formation of globular structures **(Hansma *et al.*, 1996)**. (2) Upon the melting of DNA, single-stranded DNA has the potential to undergo a coil-to-globule transition and form a globular morphology **(Ueda & Yoshikawa, 1996)**.

The reserved double-stranded structure of DNA may contain a higher number of G-C base pairs compared to the denatured regions. As the heating temperature increases, the reserved portion decreases **(Yan *et al.*, 2002)**. In this study, when the temperature was increased up to 413.15 K, a significant number of grains were observed inside the DNA chains, resembling a "beads-on-a-string" morphology (see Figure 3c). The formation of grains within the DNA structure was observed at 80 °C for AFM samples prepared by heating a solution of DNA samples **(Yan et al., 2002)**. These grains are formed because a significant part of the DNA chains was divided and then collapsed. In Figure 3d, it can be seen that the DNA chain ends with a branched section (black arrow), which could provide additional evidence for the separation of two complementary DNA chains.

At higher temperatures (443.15 K), double-stranded DNA undergoes serious degradation. This is evident from the presence of small fragments of several DNA structures with very low height (< 0.5 nm) (see Figure 3e and f). The disappearance of linear morphology and the formation of such small parts of DNA, have been noted in AFM images of DNA solutions after being heated at 100 °C **(Yan et al., 2002)**.

4- Conclusion

AFM imaging has been demonstrated as a powerful technique for monitoring DNA structural changes induced by thermal energy. AFM images of DNA samples at different temperature degrees indicate thermal melting of DNA starting from 373.15 K, showing DNA chains with globular structures. Complete denaturation of DNA strands appeared at 413.15 K, indicated by the presence of numerous grains observed inside the DNA chains. At 443.15 K, DNA molecules undergo serious degradation, resulting in the formation of small fragments with an average diameter of less than 0.5 nm, as measured by AFM cross sections.

5- References

Anabel, N., Cervantes, G. & Gutierrz-Medina, B. (2014). Robust Deposition of Lambda DNA on mica for imaging by AFM in air. *SCANNING*. 36:561-569.

Blackburn, G.M., Gait, M.J., Loakes, D. & Williams, D. (2006). Nucleic Acids in Chemistry and Biology. *Royal Society of Chemistry*. p. 586.

Bradbury, E. M., Price, W. C. & Wilkinson, G. R. (1961). Infrared studies of molecular configuration of DNA. *Journal of Molecular Biology*. 3(3):301-317.

Drew M. E., Chworos A., Oroudjev E., Hansma H. & Yamakoshi Y. (2010). A tripod molecular tip for single molecule ligand-receptor force spectroscopy by AFM. *Langmuir*. 26: 7117–7125.

Esnault C., Chnais B., Casse N., Delorme N., Louarn G. & Pilard J. (2013). Electrochemically modified carbon and chromium surfaces for

AFM imaging of double-strand DNA interaction with transposase protein. *Chem. Phys. Chem*. 14: 338–345.

Ge, D., Wang, X., Williams, K. & Levicky. R. (2012). Thermostable DNA Immobi-lization and Temperature Effects on Surface Hybridization. *Langmuir*. 28(22): 8446-8455.

Hansma H. G., Kasuya K. & Oroudjev E. (2004). Atomic force microscopy imaging and pulling of nucleic acids. *Curr. Opin. Struct. Biol*. 14: 380–385.

Hansma, H. G., Revenko, I., Kim K. & D. E. Laney. (1996): Atomic force microscopy of long and short double-stranded, single-stranded and triple-stranded nucleic acides. *Nucl. Acids Res*. 24(4). 713-720.

Houlton, A. & Watson, S.M.D. (2011). DNA-based nanowires. Towards bottom-up nanoscale electronics. *Annu. Rep. Prog. Chem. Sect*. A,. 107: p. 21-42.

Jin, J. I. & Grote, J. (2011). Materials Science of DNA, 1 ed., CRC Press. Florida. USA.

Kudo, H. & Fujihira, M. (2006). DNA-templated copper nanowire fabrication by a two-step process involving electroless metallization. *Nanotechnology, IEEE Transactions on*. 5(2): 90-92.

Lee, S. Debenedetti, P. Errington, J. Pethica, B. & Moore, D. (2004). A Calorimetric and Spectroscopic Study of DNA at Low Hydration. *J. Phys. Chem. B*. 108(9): 3098–3106.

Lyubchenko Y. L., Gall A. A. & Shlyakhtenko L. S. (2014). Visualization of DNA and protein-DNA complexes with atomic force microscopy. *Methods Mol. Biol*. 1117: 367–384.

Mohamed, H.D.A., Watson, S. M. D., Horrocks, B.R., & Houlton, A. (2015). Chemical and electrochemical routes to DNA-templated rhodium nanowires. *Journal of materials chemistry C*. 3(2): 438-446.

Mohamed, H.D.A., Watson, S.M.D. Horrocks, B.R. & Houlton, A. (2012). Magnetic and conductive magnetite nanowires by DNA-templating. *Nanoscale*, 2012. **4**(19): 5936-5945.

Niziol, J., Nowak, P., Kobierski, J. & Haranczyk, H. (2015). Temperature evolution of hydration shells in solid DNA didecyldimethylammonium chloride complex studied by 1H NMR spectroscopy. *Eur. Polym. J*. 66: 301–306.

Niziol, J., Ekiert, R., Kuczkowska. J., Fryn, P., & Marzec, M.(2019). Thermal degradation of biological DNA studied by dielectric spectroscopy. *Polymer Testing*.80:106158.

Pang, D., Thierry, A. R. & Dritschilo, A. (2015). DNA studies using atomic force microscopy: capabilities for measurements of short DNA fragments. *Molecular Biosciences*. 2(1).

Panigrahi, S. Pal, R. & Bhattacharyya, D. (2011) Structure and energy of non-canonical basepairs: comparison of various computational chemistry methods with crystallographic ensembles. *J. Biomol. Struct. Dyn.* 29: 424–596.

Rugar, D. & P. Hansma. (1990) Atomic force microscopy. *Physics Today*. 43(10): 23-30.

Sebastiani, F., Pietrini, A., Longo, M., Comez, L., Petrillo, C. & Sacchetti, F. (2014). Paciaroni, Melting of DNA nonoriented fibers: a wide-angle X-ray diffraction study. *J. Phys. Chem.* B. 118:3785–3792.

Stryer, L. (1988). Biochemistry. 3rd ed.W. H Freeman & Co. 1089. New York, USA.

Ueda, M. & Yoshikawa K. (1996). Phase Transition and Phase Segregation in a single Double-stranded DNA molecule. *Phys. Rev. Lett*. 77(10): 2133.

Wang, L. Yoshida, J. & Ogata, N. (2001). Self-assembled supramolecular films derived from marine deoxyribonucleic acid (DNA) cationic surfactant complexes: large-scale preparation and optical and thermal properties. *Chem. Mater.* 13(4):1273–1281.

Watson, S. M. D., Mohamed, H.D.A., Horrocks, B.R., & Houlton, A. (2013). Electrically conductive magnetic nanowires using an electrochemical DNA-templating route. *Nanoscale*. 5(12): 5349-5359.

Xu, M., Dai, T., Wang, Y. & Yang, G. (2022). The incipient denaturation mechanism of DNA. Royal Society of Chemistry. (12):23356-13365.

Yan, L. & Iwasaki, H. (2002). Thermal Denaturation of Plasmid DNA Observed by Atomic Force Microscopy. *Japanese Journal of Applied Physics*. 41(12):7556-7559.

Yao, N. & Wang, Z.L. (2005). Handbook of Microscopy for Nanotechnology. Springer London, Limited.