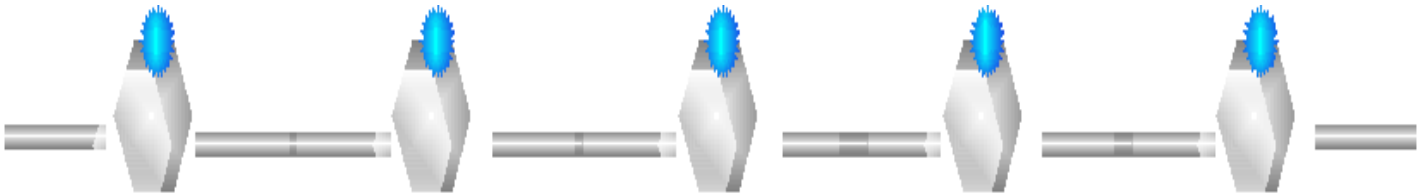
CHALMERS



Assembly and Characterization of Circular DNA Nanostructures

Master of Science Thesis

CHARLOTTA FREDRIKSSON

Department of Chemical and Biological Engineering
Division of Physical Chemistry
CHALMERS UNIVERSITY OF TECHNOLOGY
Gothenburg, Sweden, 2010

THESIS FOR THE DEGREE OF MASTER OF SCIENCE

Assembly and Characterization of Circular DNA Nanostructures

Charlotta Fredriksson

Department of Chemcial and Biological Engineering

CHALMERS UNIVERISTY OF TECHNOLOGY

Gothenburg, Sweden, 2010

Assembly and characterization of circular DNA nanostructures

Department of Chemical and Biological Engineering
Chalmers University of Technology
SE-412 96 Gothenburg
Sweden
Telephone + 46 (0)31-772 1000

Cover: Illustrative image of linked DNA-rings with cyanine-5 modification on one oligonucleotide. Note that this is only an illustrative image of the DNA-ring, it is not a 3D structure in real life.

Department of Chemical and Biological Engineering
Gothenburg, Sweden, 2010

Assembly and characterization of circular DNA nanostructures

Charlotta Fredriksson

Department of Chemical and Biological Engineering
Chalmers University of Technology

Abstract

Addressable molecules such as DNA, and also to some extent RNA, are useful as nanomaterials. Both of these molecules have well known structures, which can be used to form non-repetitive, small and high-informative nanonetworks. These networks can be formed by using sequences of oligonucleotides that are designed to be complementary in a predetermined pattern, which can give rise to functionalized DNA nanoconstructs such as circular DNA-nanoconstructs, i.e. DNA-rings. These DNA constructs can hopefully be used in e.g. intelligent drug delivery and programmable chemical synthesis.

This Master's thesis focuses on two aspects of DNA-ring formation: 1) the effect of cyanine dyes on DNA-ring formation and 2) the formation of linked DNA-rings. The first part will use two cyanine fluorophores (Cy3 and Cy5) to visualize the DNA-rings and study their effect on the formation of the DNA-rings. Also, the time and temperature of hybridization of the DNA-rings will be studied to observe how this affects their formation. The second part will focus on the formation of extended DNA-rings with links, and try to polymerize these for potential use in modular build-up of nanonetworks.

Results from the first part have suggested that the fluorophores do affect formation, especially the formation of the 6 mer and 12 mer DNA-ring. The former seems to increase the less number of Cy while the latter decreases the less number of Cy. The time and temperature of hybridization also affect formation, a higher temperature seems to decrease the formation of larger DNA-rings, while a lower temperature seems to increase the formation of larger rings. Different hybridization times, on the other hand, seem to give a constant formation of both small and large DNA-rings, and this seems to apply to both Cy5 and Cy3 modified DNA-rings.

The second part of the project, dealing with linked DNA-rings has shown that linked DNA-rings can be formed and that polymerization of DNA-rings may be possible. It is unclear if polymerization of the linked DNA-rings can be performed but results have indicated that it may be possible. The stoichiometry of Glue can be an explanation of the inefficient polymerization of linked DNA that has been presented in the results of this project.

Keywords: DNA nanotechnology, addressable molecular node assembly (AMNA), gel electrophoresis, DNA-rings, cyanine dyes.

List of abbreviations

AMNA	Addressable Molecular Node Assembly
A	Adenine
DNA	Deoxyribonucleic acid
C	Cytosine
Cy3	Indocarbocyanine-3
Cy5	Indocarbocyanine-5
FRET	Fluorescence (or Förster) resonance energy transfer
G	Guanine
NNA	Nearest-neighbor approximation
RNA	Ribonucleic acid
T	Thymine

Table of contents

1. Introduction

The development of nanostructures that can be used for e.g. intelligent drug delivery and programmable chemical synthesis is an interesting and developing field in nanotechnology.[1] In nanotechnology, it is essential to produce networks with small building blocks to get small but yet informative nanostructures. It is also important to enable thermodynamically stable and rigid networks, without the interfering of byproducts. Therefore, the study of thermodynamic and kinetic aspects of nanomaterial assembly is an important research area.[2]

Recent research in nanotechnology has focused on using DNA as nanomaterial; this is because DNA provides high rigidity, high binding specificity, high information density and a well known structure. By choosing DNA oligonucleotides that are designed to form DNA nanoconstructs in a specific pattern, one can form non-repeating and functionalized networks.[3] Also, DNA is easy to visualize with the help of fluorophores, which enables studies of the nanoconstructs with e.g. fluorescence resonance energy transfer (FRET). Although, studies have shown that fluorophores can affect the formation of nanoconstructs and their thermal stability. Therefore it is important to investigate this issue further.[2]

Since DNA nanotechnology studies have shown that DNA-rings are probably the smallest existing nanoconstruct that can be used to build nanonetworks, it is important to study the DNA-ring. Therefore this project will study the formation of the DNA-ring and how this is affected by fluorophores, and the time and temperature of hybridization.

Also, since it is important to be able to build larger networks it is interesting to investigate extended DNA-rings, which are formed by oligonucleotides that link together the DNA-rings. Therefore this master thesis project will study the formation of linked DNA-rings and try to polymerize them, so that larger networks can be formed.

Thus, this project focus on studying two aspects in DNA-ring formation: 1) the effect of cyanine dyes on DNA-ring formation and 2) the formation of linked DNA-rings.

2. Theory

2.1. DNA

DNA is a negatively charged macromolecule, in which nucleotides bind to each other covalently. The nucleotides consist of a sugar, a phosphate group and nucleobases which bind to each other via Watson Crick base-pairing (see fig 2.1).[4] This kind of base-pairing is a specific and complementary binding, where adenine binds to thymine (A-T) and guanine binds to cytosine (G-C). The specific binding of the bases and the well known rigid structure of DNA makes it a good candidate for nanomaterial.[3]

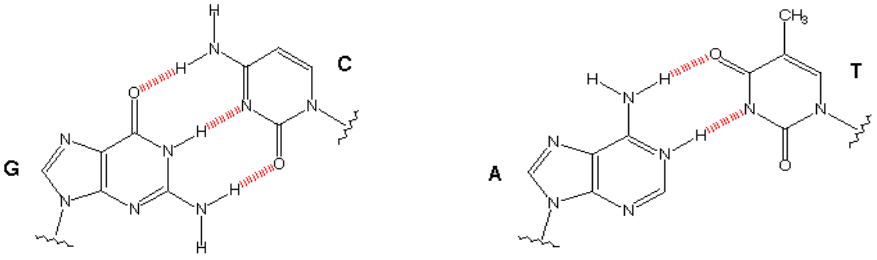


Fig 2.1. Watson and Crick basepairing; G-C binding (to the left) and A-T binding (to the right).[4]

Also, DNA has the ability to incorporate a third strand in the normal Watson-Crick base paired duplex by Hoogsteen base-paring in the major groove, forming a triplex.[3]

The DNA molecule and DNA constructs can be studied with fluorescence techniques by labeling the DNA strands with fluorescent molecules, i.e. fluorophores. Fluorescent probes are therefore useful when visualization and detection of DNA is needed. Molecules can bind to DNA by intercalation or in the minor or major groove (see fig 2.2).[10, 16]

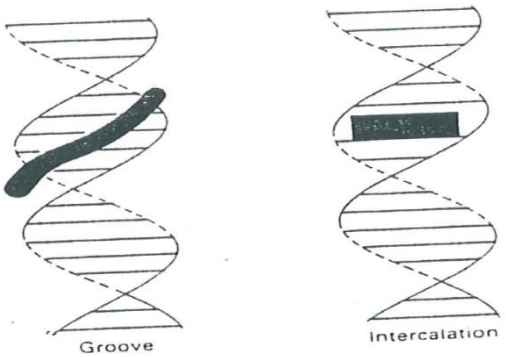


Fig 2.2. Binding to DNA by groove binding (to the left) and intercalation (to the right).[10]

An additional DNA binding mode is electrostatic attraction, in which positively charged ions attract to the negatively charged DNA backbone.[16]

2.2. DNA nanotechnology

The field of nanotechnology has two main areas referred to as the "top down" and the "bottom up" approach. Recent research in nanotechnology has been more focused on the "bottom up" approach, meaning that small molecules are used to form larger structures. The present advances in nanotechnology are striving towards the goal that one should use smaller structures with a high information content to form larger highly informative networks.[3]

To achieve this, DNA has been used in so called DNA nanotechnology. DNA is useful in nanotechnology since it self assembles according to the Watson & Crick base pairing.[1] This self assembly property can be controlled by choosing the design of the base sequence, which is a useful property in nanotechnology. DNA nanotechnology has been used to form DNA nanomachines that can be used for molecular sensing, intelligent drug delivery and programmable chemical synthesis.[1] Research in DNA nanotechnology has also shown that cubes, truncated octahedrons (fig 2.3), 2-D and 3-D crystals can be formed with DNA.[7, 17]

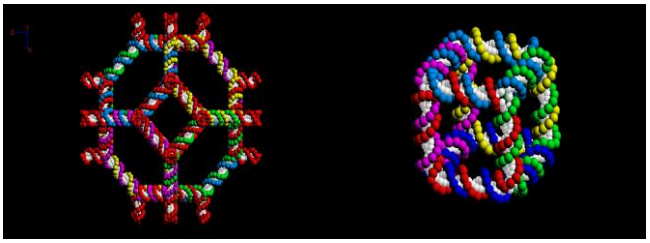


Fig 2.3. Truncated DNA octahedron (to the left) and DNA cube (to the right).[20]

The advantages of using DNA in nanotechnology are the fact that is has a highly selective bonding of the nucleobases and a steric stiffness due to the double-helical structure. Also, repeating networks of DNA provide stability and rigidity. However, they increase the size of the unit cell and it is therefore essential to develop non-repeating networks based on smaller unit cells. These non-repeating networks can be obtained by using short and highly informative building blocks of DNA i.e. oligonucleotides. By using sequences of oligonucleotides that are designed to be complementary in a decided pattern, one can form functionalized DNA nanoconstructs, e.g. DNA-rings (see fig 2.4).

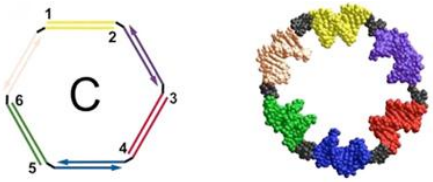


Fig 2.4. Image of DNA-ring, where 1 to 6 labels each of the oligonucleotide types and the color shows which oligonucleotide strands that are complementary to each other.[3]

By taking advantage of addressable molecules as DNA to form so called addressable molecular node assembly, abbreviated AMNA, one can form these kinds of nanoconstructs. One potential application of the AMNA project is to form extensive two dimensional nanonetworks with functionalisation at specific sites in the network to be able to perform energy transfer in a predetermined pattern.[6]

One part of the AMNA project is to form networks with the help of synthetic three way oligonucleotides, i.e. branched oligonucleotides. So far a network of two adjacent hexagonal unit cells (DNA naphthalene) has been published.[5]

The unit cell in the AMNA project is a DNA hexagon (DNA-ring) which is possibly the smallest nanoconstruct existing, the side length is only that of one turn of DNA and the basic component size is 10 bases.[3] The study of DNA nanoconstructs is easily managed and visualized with the help of fluorophores that bind to DNA in major or minor groove, or by intercalation. DNA can also be modified at different sites on the strand; one example is to modify the DNA at the 5'-terminus end with a cyanine dye, usually referred to as Cy-modification.[8]

2.3. Fluorescence

When a molecule absorbs energy, it exists a number of routes by which the energy can return to the ground state of the molecule. One of these routes is fluorescence, which is a process where a photon is emitted when the molecule relaxes from the first singlet state (S1) state to the ground state (S0), normally (see fig 2.5).[9]

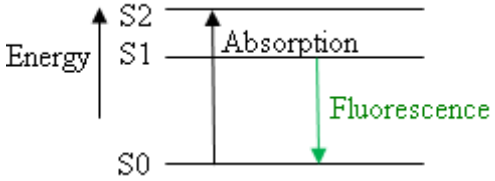


Fig 2.5. Absorption and fluorescence of an arbitrary molecule.

To be able to study molecules as DNA, the use of fluorescent probes is necessary since DNA itself is non-emissive. DNA can for example be covalently modified with a fluorophore. One example of this is the cyanine (Cy) modification, where the fluorophore is covalently attached to DNA, e.g. to the 5'-terminus end.[8]

Fluorophores enables visualization of the molecule but can also be used to measure distances indirectly with so called fluorescence resonance energy transfer (FRET) studies. FRET is a technique in which one can measure distances depending on the degree of energy transfer between two fluorophores. By labeling e.g. nanoconstructs with fluorophores on specific positions, the structure can be deduced. Since fluorescence is highly sensitive, FRET has become a widely used technique in studies of molecules and in nanotechnology. It has for example been used to measure the size and length of nanoconstructs.[3]

2.3.1. Cyanine fluorophores

Cyanine dyes are a group of fluorescent molecules that can function as DNA probes, so that DNA can be studied with help of fluorescent techniques. Cyanine donor – acceptor fluorophore pairs has especially become popular in single-molecule FRET studies.[8]

One example of a cyanine donor –acceptor FRET pair, is the indocarbocyanine-3 (Cy3) and the indocarbocyanine-5 (Cy5) donor-acceptor pair. When Cy3 or Cy5 (fig 2.6) is attached onto the end of the DNA helix it stacks on the bases, in a similar way to that of an additional base pair. This is commonly referred to as the Cy-modification which is useful when visualization of DNA is desirable [8, 11]

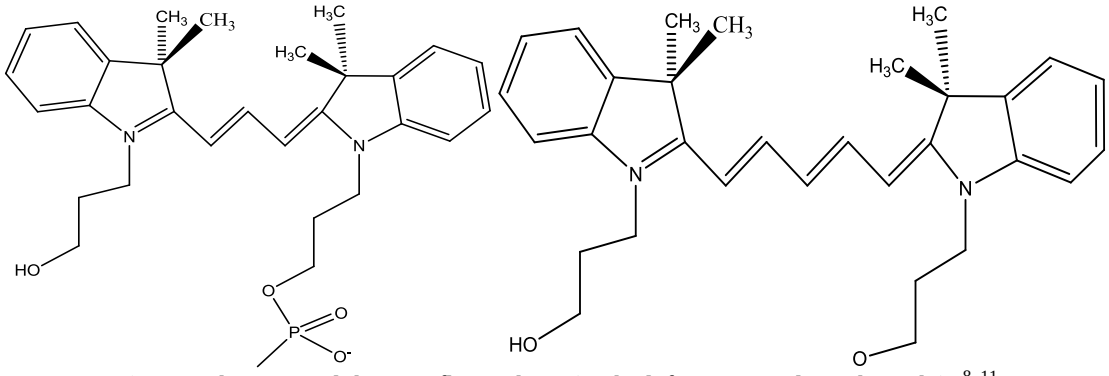


Fig 2.6. The Cy3 and the Cy5 fluorophore (to the left respectively to the right). [8, 11]

In the AMNA research, Cy3 and Cy5 have been used to visualize DNA constructs by using Cy-modified oligonucleotides.[6] Some of these studies have suggested that the Cy-modification can affect the formation and size of the DNA-rings under certain conditions. Therefore, it is important to perform further experiments with cyanine modified oligonucleotides to study their effect on DNA-rings.[2]

Other studies on fluorophores have shown that they can have an effect on the thermodynamic stability and that both fluorophore- fluorophore and fluorophore-DNA interactions can be detected when studying e.g. DNA-duplexes. Also, additional fluorophores which are attached to the end of DNA can contribute stability to the duplex, by stacking like an additional base pair. Studies have shown that Cy5 and Cy3 increase the melting temperature of DNA and stabilize the construct.[15]

2.4. Gel electrophoresis

Electrophoresis is a method commonly used to separate molecules by size and charge. For example, in biochemistry it is used to separate molecules such as DNA, RNA and proteins. The separation of molecules is achieved by applying a constant or pulsed electric field over a gel that is loaded with the molecule of interest. The molecule will then move through the pores of the gel according to the size and charge. The gel that is most commonly used consists of a rigid polysaccharide polymer such as agarose.[12] The agarose will form a gel with pores, in which molecules will need to pass through. The passing of spherical, small molecules through these pores can be explained by the Ogston theory. The theory states that spherical molecules will travel differently through the gel depending on the percentage of polymer in the gel; a higher percentage yields smaller pores which makes the mobility of molecules slower.[13, 14] Since the DNA-rings are rather small and ring formed, the Ogston theory is likely to explain the passing of DNA-rings through the gel. A linear long molecule on the other hands is more likely to be explained by the reptation theory, in which the molecule migrates like a reptile through the gel.

When negatively charged molecules such as DNA are studied they will move toward the anode according to their size, meaning that large DNA fragments will travels slower than small DNA fragments.[14] To visualize the separation of DNA fluorescent dyes are used. These are either attached directly to the DNA by covalent modification e.g. to the 5'-terminus end or bind via intercalation or in major or minor groove. By using a gel scanner the intensity of each DNA band can be measured with help of a computer software program.

2.5. UV melting of DNA

Single stranded DNA (ssDNA) has higher absorbance than double stranded DNA (dsDNA) at 260 nm, this is due to that dsDNA has hydrophobic π-stacking between the aromatic bases which give rise to a lower molar extinction coefficient at the wavelength 260 nm (ε_{260}). This phenomenon enables monitoring of the denaturation process of dsDNA by using absorption spectroscopy and the melting temperature (T_m) of an oligonucleotide can be determined by varying the temperature and continuously measuring the absorbance.

3. Experimental

3.1. DNA rings with cyanine modifications

The material used was twelve different 22 mer oligonucleotides. These consisted of ten bases, two unpaired TT bases and lastly ten more bases. Three different types of oligonucleotides were used, one type was unmodified, and two other were Cy3 and Cy5 modified in 5'- end, respectively. The 22 mer oligonucleotides are designed to be complementary to each other in a determined pattern (see fig 3.1), so that the DNA-ring is formed.

Fig 3.1. Design of DNA-ring, where H1 to H6 labels each of the oligonucleotide types, and the color shows which oligonucleotide strands that are complementary to each other.

A linear DNA construct will also be produced for comparison with the DNA-rings. The linear DNA will be produced by using an oligonucleotide with one side of only thymines (H6T), thus making one side non-complementary to its neighboring oligonucleotides and unable to ring-close. Table 1 shows each oligonucleotide that was used for the formation of hexagonal and linear DNA.

Table 1. Sequence of oligonucleotides for DNA-ring.

Oligonucleotide	**Sequence**
H1	5' CCA TAC ATA C TT CCA CAG CAT C 3'
H2	5' GGC TCT ACA G TT GAG GAG GAT G 3'
H3	5' GAT TAG CGT G TT CGA TGG TAT C 3'
H4	5' GAC GCT AAT C TT GAT GCT GTG G 3'
H5	5' GTA TGT ATG G TT CAT CCT CCT C 3'
H6	5' CTG TAG AGC C TT GAT ACC ATC G 3'
H6T	5' CTG TAG AGC C TT TTT TTT TTT T 3'

3.2. Linked DNA-rings

DNA-rings with extensions will also be produced by replacing H1 and H6 with oligonucleotides that are called “Link 1” and “Link 2” respectively (see fig 3.2).

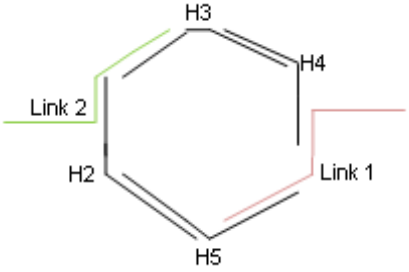


Fig 3.2. Design of DNA-ring with links.

The extended DNA-rings will be used to form DNA-rings that are linked together, as seen in figure 3.3. The linking between them will be performed with the help of an oligonucleotide called “Glue”, which is complementary to the protruding part of Link 1 and Link 2.

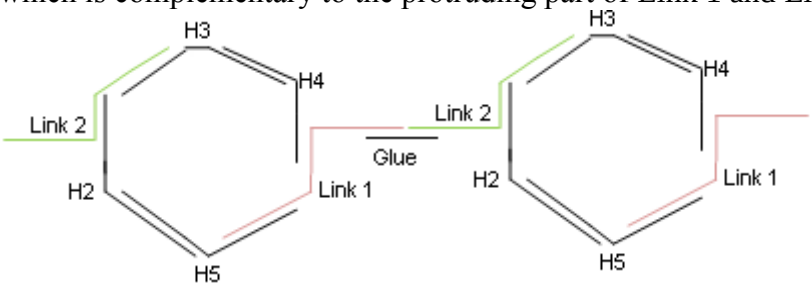


Fig 3.3. Linked DNA-rings.

When two linked DNA rings are connected the product is called biphenyl DNA. It is called biphenyl DNA since the structure resembles that of the molecule biphenyl. Scheffler et. al. introduced this kind of notation for DNA nanoconstructs. By conceiving a single-stranded oligonucleotide as an electron and a double-stranded oligonucleotide as a C-C bond, DNA nanoconstructs are named after their counterparts in chemistry.[19]

Table 2. Sequence of links, Glue and complementary 10 mer oligonucleotides.

Oligonucleotide	**Sequence**
Link 1	5’ CCA TAC ATA C TT CCA CAG CAT CTT CAG CTT GAG G 3’
Link 2	5’ GTG TGA GAA G TT CTG TAG AGC C TT GAT ACC ATC G 3’
Glue	5’ CTT CTC ACA C CCT CAA GCT G 3’
1’	5’ CCT CAA GCT G 3’
6’	5’ CTT CTC ACA C 3’

Additional oligonucleotides used were two 10 mer oligonucleotides: 1’ and 6’ (see table 2 above) that where complementary to Link 1 and Link 2 respectively. These oligonucleotides were used to form DNA-rings with 10 additional bases, so called 10 mer DNA-ring (fig 3.4).

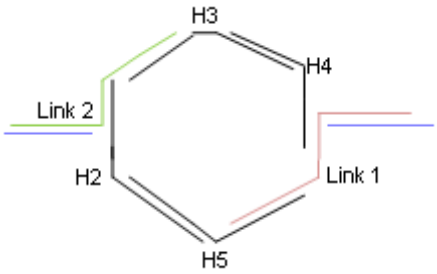


Fig 3.4. 10 mer DNA-rings, with 1’ and 6’ shown in purple.

3.2.1. Polymerization of linked DNA-ring

Since linked DNA-rings can be connected to each other with the help of the Glue oligonucleotide, it is essential to explain how this connection can be performed. The connection of many DNA-rings will be referred to as polymerization.

The polymerization of linked DNA can be carried out in two ways, the Glue is either added to samples containing DNA-rings with both links, or added to samples that contain a mixture of the DNA-ring with Link 1 and the DNA-ring with Link 2. In the first case, it can theoretically form a linking of many DNA-rings, while it can only form a linking of two DNA-rings in the second case (see fig 3.5), so called biphenyl.

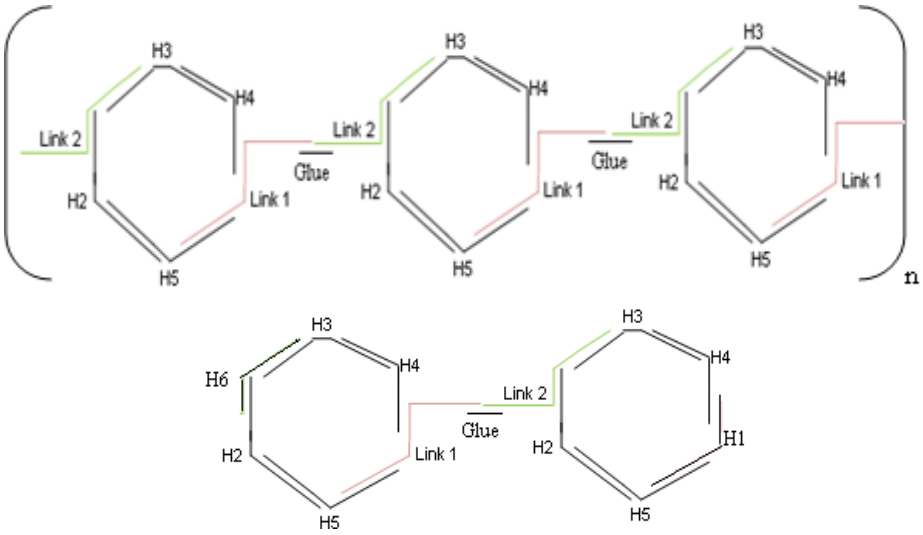


Fig 3.5. Polymerization of DNA-rings
(Above: DNA-ring with both links. Below: DNA-ring with only one link).

The results of the different polymerizations will be presented in chapter 4.2.2.

3.3. Sample preparation

Each sample of oligonucleotide was prepared with filtrated phosphate buffer to a concentration of 2 μM set by the absorption at 260 nm using Beer-Lamberts law:

$$A = \varepsilon c l$$

where A is the absorption, c is the concentration, ε is the molar extinction coefficient ($M^{-1}cm^{-1}$) and l is the length of the cuvette (cm). The molar extinction of each sequence had been calculated previously using the nearest-neighbor approximation (NNA).

All DNA constructs were prepared by equimolar mixing of the oligonucleotides and each prepared sample was then hybridized.

3.4. Hybridization

Since the time and temperature of the hybridization affects the formation of the nanoconstructs, these parameters were varied for the different experiments.

The temperature was varied by heating up the samples to 90 °C and then letting them cool down to the desirable temperature. The temperature range was 90 °C – 25 °C, and the heating of the samples was performed with two heating blocks (QBH2 Grant and QBT1 Grant). The two heating blocks were compared to each other to see if they affected the formation differently (see appendix). The temperature of the hybridization was also varied by using a computer assisted water bath, in which the samples were heated to 85 °C and then cooled down to 5,5 °C over three hours, with a linear gradient. All temperature results can be seen in chapter 4.1.3.2 and 4.1.4.2 for the Cy3 and Cy5-modified DNA-ring, respectively.

Time was varied by using a fixed temperature of the hybridization and the samples were taken out from the heating block at different times. The time range was 20 – 140 min with 20 min intervals and results are shown in 4.1.4.1 and 4.1.3.1 for the Cy3 and Cy5 respectively.

3.5. Gel electrophoresis

The nanoconstructs were separated and analyzed using gel electrophoresis. The protocol used was with a gel of 4,5 % Metaphor agarose from Tebu-bio, a voltage of 67, a field strength of approximately 4 V and with ficoll (10% w/w) as the loading buffer. The running buffer was 109 mM phosphate buffer ($[Na^+]$ = 200 mM, pH 7,5).

Each gel was scanned using a gel scanner (Typhoon 9610 GE Healthcare) and the bands were indentified according to the distance they migrated in the gel.

The intensity of each band was analyzed with the help of a program called Image Quant. Each band was selected with the program (se fig 3.6), and was corrected with respect to the background.

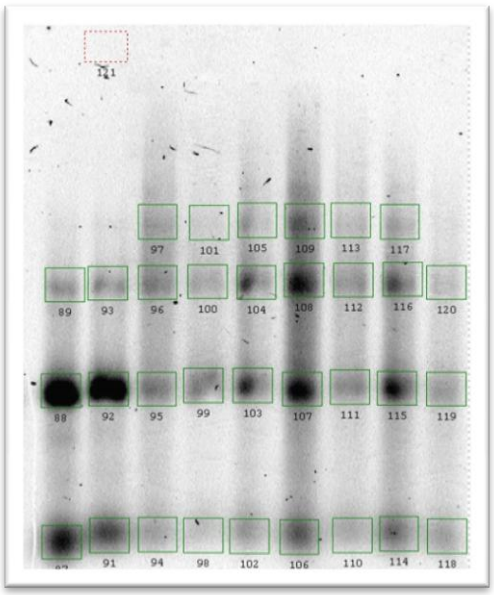


Fig 3.6. Selecting the bands for intensity measurements (green squares) and the background subtraction (red square). (From bottom: monomer-, 6 mer-, 12 mer- and 18 mer band).

The total intensity of each sample was taken as the sum of all bands, and the ratio of the band intensity and the total intensity was calculated to get the yield of formation. The yield was then used for comparison between the different experiments.

3.6. Melting temperature

The melting temperatures for DNA-ring with and without Cy5 modification and linked DNA-ring with both links with and without Glue were collected by performing melting curve experiments with a Cary 4000 UV-Vis spectrophotometer.

Each sample was heated from 5,5 °C to 90 °C with a 0,5 °C increase per minute. After heating, the samples were cooled down to 5,5 °C. This procedure was performed four times for each sample and the melting curves were collected i.e. two heating steps and two cooling steps. Each curve was normalized for better visualization and the derivative of each curve was calculated. By locating the maximum point of the derivate, the melting point (T_m) could be estimated. The T_m presented is the average T_m for all four steps. The melting curves and their derivates will be presented for both the heating and the cooling step.

4. Results

4.1. The effect of cyanine modification on DNA-ring size

4.1.1. Ladder with increasing number of oligonucleotides

To prove that DNA-rings could be formed, experiments were performed with Cy3 modified oligonucleotides, so that the samples contained one additional oligonucleotide of the DNA-ring in each sample. Sample 1 will therefore only contain a monomer (the H3 oligonucleotide), sample 2 will contain a dimer (H3 and H4) and so on, until sample 6 which contains all six oligonucleotides, thus hopefully forming a complete DNA-ring (see table 3). Sample 6-8 contain the hexagon and sample 9 contains linear DNA, so that one can compare these with the rest of the samples.

Table 3. Sample of ladder.

Sample	**Oligonucleotide**
1. Monomer	H3
2. Dimer	H3, H4
3. Trimer	H3, H4, H1
4. Butamer	H3, H4, H1, H5
5. Pentamer	H3, H4, H1, H5, H2
6-8. Hexagonal (6 mer) DNA	H3, H4, H1, H5, H2, H6
9. Linear DNA (6 mer)	H3, H4, H1, H5, H2, H6T

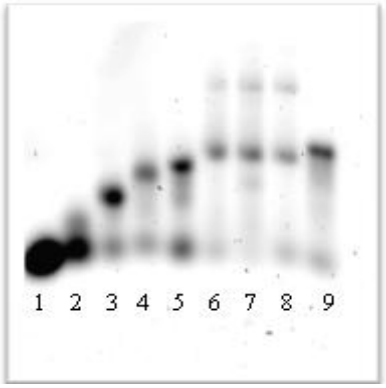


Fig 4.1. Gel from ladder experiment. (From left: sample 1 to 9).

As seen in figure 4.1. the samples migrate shorter distance the more oligonucleotides added, thus proving that larger constructs are formed. Sample 1 has the longest migration while sample 9 has the shortest. Since the linear DNA can only form a 6 mer long linear construct, this should yield one band, the 6 mer band. As seen in fig 4.1, sample 9 (linear DNA) shows only one band as excepted. If 6 mer DNA-rings are formed, sample 6-8 should show bands that migrate differently compared to the band in sample 9, since the 6 mer ring should migrate differently than the linear 6 mer construct. The difference in mobility between the circular and the linear constructs is explained by the difference in shape, much like the difference between the Ogston and the reptation theory. Therefore the circular constructs could be said to migrate according to the Ogston theory while linear could be explained by the reptation theory. Although is hard to say if this is the case since they are very small constructs.

As seen in fig 4.1, bands are observed in sample 6-8 at different position than in sample 9, thus indicating that these samples contained a ring closed 6 mer construct i.e. the 6 mer DNA-ring. The highest band in sample 6-8 most likely contains a ring closed 12 mer because of the large difference in mobility compared to the ring closed 6 mer. The only samples that show bands above the 6 mer band, are the samples that contain the DNA-rings, it is suggested that 12 mer DNA-rings have been formed. These bands have migrated a shorter distance than the 6 mer ring closed structure and the linear constructs thus indicating that these samples contain constructs that are larger than six oligonucleotides (6 mer). If it was to be a linear construct it should form many bands due to polymerization. Also one should recognize the small difference in mobility when comparing linear constructs of similar size, e.g. comparing sample 4 and 5. It should be noted that previous studies have shown that ring sizes of multiples of 6 can be shown and therefore with these results in hand one know that the DNA-ring can form 12 mer, 18 mer and so on.

4.1.2. Ladder with decreasing number of Cy3

To see whether the Cy3 modification has any effect on the formation of DNA-rings, experiments with different numbers of Cy3 in the DNA-ring were performed. In these experiments, the samples were prepared by using modified and unmodified oligonucleotides, so that DNA-rings were formed with decreasing number of Cy3 (see table 4).

Table 4. Sample for ladder with decreasing Cy3.

Sample	**Unmodified oligonucleotides**	**Cy3 modified oligonucleotides**
1.		H1, H2, H3, H4, H5, H6
2. Replicate sample of 1		H1, H2, H3, H4, H5, H6
3.	H3	H1, H2, H4, H5, H6
4.	H3, H4	H1, H2, H5, H6
5.	H3, H4, H1	H2, H5, H6
6.	H3, H4, H1, H5	H2, H6
7.	H3, H4, H1, H5, H2	H6

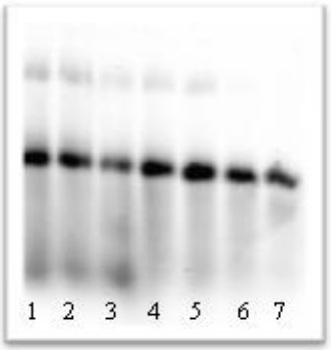


Fig 4.2. Gel image of the ladder with decreasing number of Cy3.
(From left: sample 1 to 7).

No formation was detected for the monomer or the 12 mer, for the samples that contained two Cy3 and one Cy3 (sample 6 and 7), and therefore these samples will not be presented in fig 4.3 below. The two samples containing the DNA-ring with all six oligonucleotides Cy3 modified is shown as an average yield.

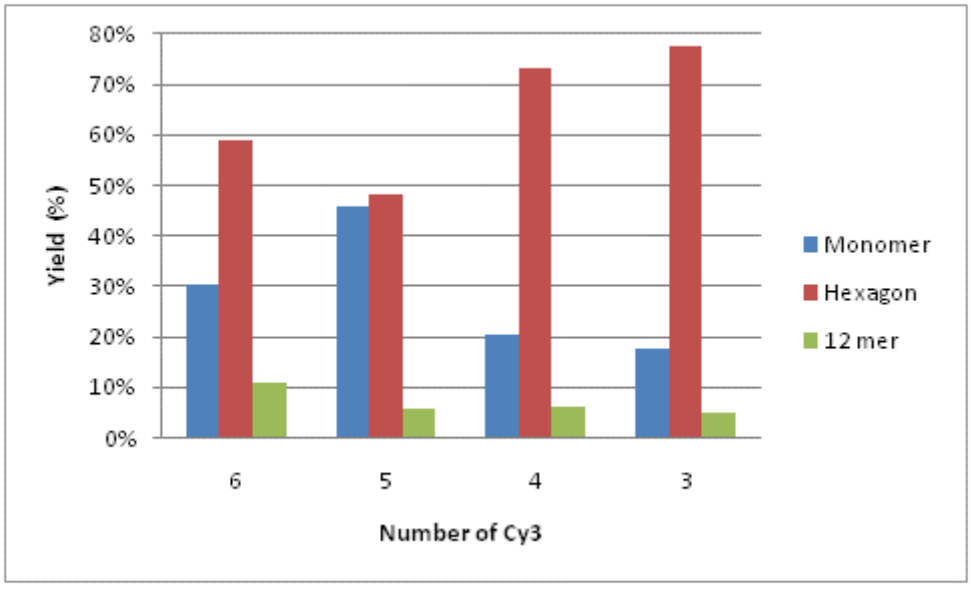


Fig 4.3. Intensities for each sample.

As seen in fig 4.3, the 12 mer formation decreases the less Cy3, while the 6 mer formation seems to increase the less Cy3. The formation of 12 mer is low, but increases the higher number of Cy, which indicates that a higher number of cyanines increases the formation of the larger rings.

4.1.3. DNA-ring with Cy3 modification

All time and temperature studies were performed on DNA-rings, in which all oligonucleotides were modified with Cy3 at the 5'-terminus end.

4.1.3.1 Time studies

The time study was performed so that each sample was taken out of a heating block (QBT1 Grant) at different times with a 20 min interval, while the temperature was held constant at 50 °C. This temperature was chosen since the temperature studies for Cy3 have indicated that it exists a maximum of large ring formation at 50-55 °C (see chapter 4.1.3.2).

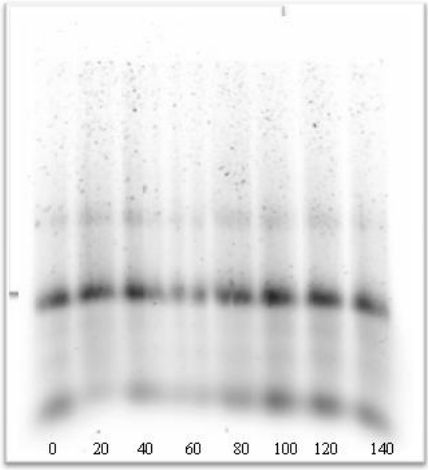


Fig 4.4. Gel image from time experiments with each lane labeled with respective time in min. (From left: 0 to 140 min).

As seen in fig 4.5, the yield of the monomer is high while the 12 mer formation is quite low. This suggests that a high yield of monomer inhibits the formation of larger rings, i.e. the monomer stoichiometry affects the stoichiometry of the larger rings. This indicates that the DNA-ring system is quite sensitive to stoichiometry of the different DNA-ring sizes. Also the low formation of larger rings (12 mer and 18 mer) could be due to that the monomer and/or other DNA-ring sizes quench the formation of these.

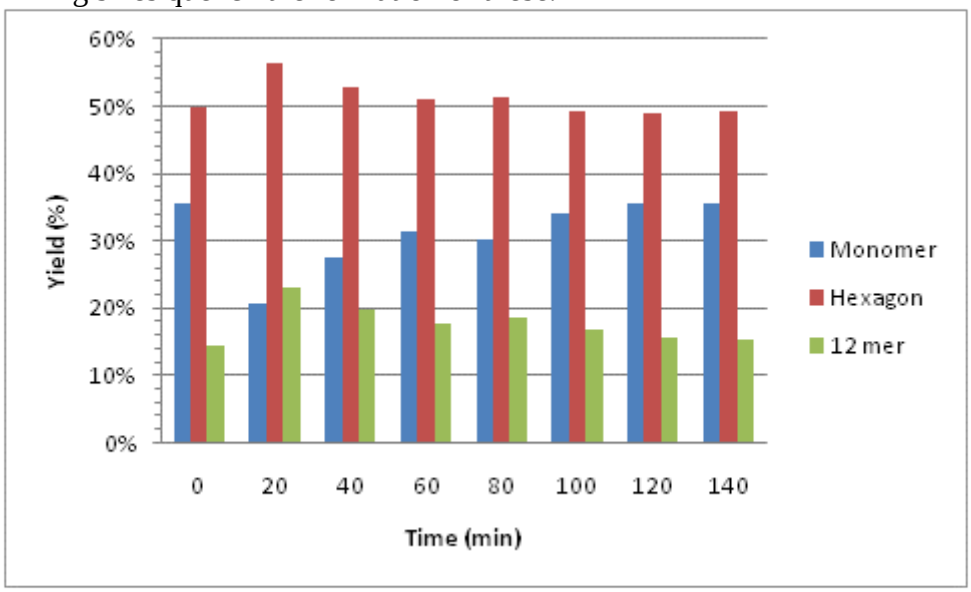


Fig 4.5. Collected intensities for each band type for the time studies.

It is also observed that the yield of monomer seems to increase with time while the 12 mer and hexagon seem to decrease with time.

4.1.3.2. Temperature studies

The hybridization was done by heating the samples in a heating block (QBT1 Grant) to 90 °C, which then was turned off and samples were taken out at specific temperatures. Fig 4.6 shows the gel from the temperature experiment and each lane is labeled with the temperature for each sample.

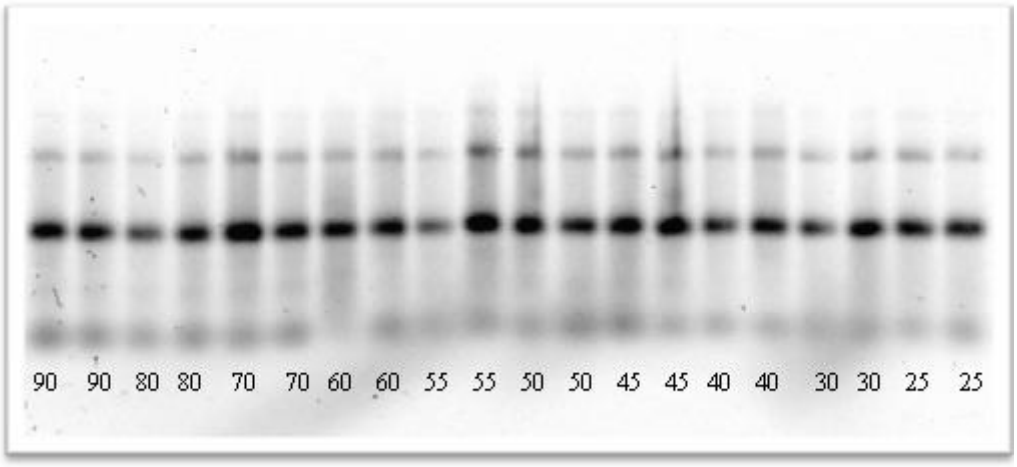


Fig 4.6. Gel image for temperature experiments with each lane labeled with respective temperature. (From left 90 to 25 °C).

Figure 4.7 shows the average intensity for each band type: monomer, 6 mer (hexagon), 12 mer and 18 mer, for all temperatures that were tested.

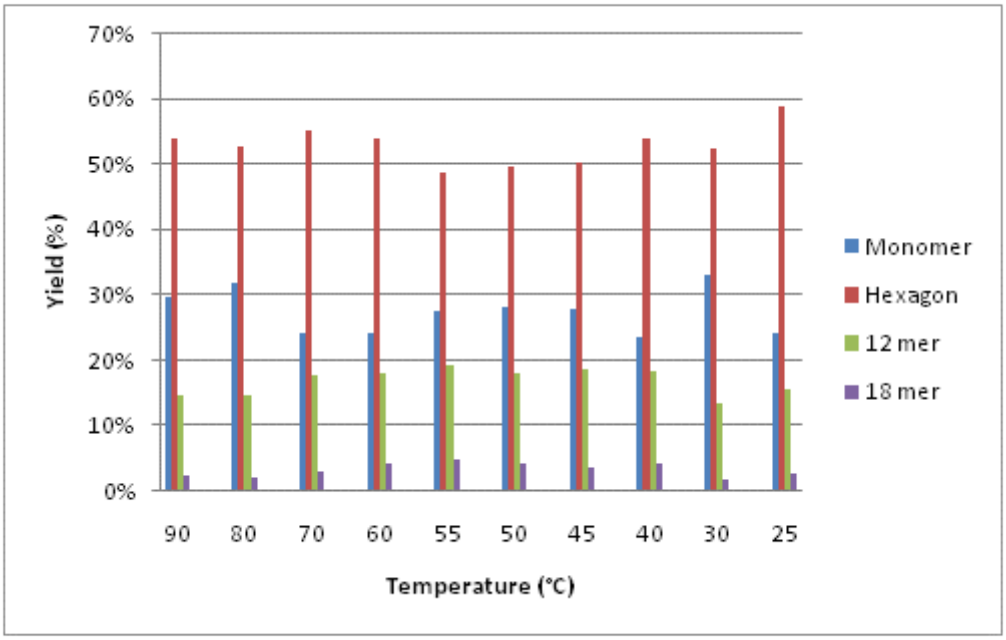


Fig 4.7. Collected intensities for each band type for the temperature studies.

As seen, no direct trend can be observed for the different ring sizes. Although it seems like the hexagon formation is quite high while the formation of 12 mer and 18 mer is low. The 12 mer and 18 mer seem to have a maximum at 50-55 °C. The temperature 50 °C was therefore studied in the time experiments.

4.1.4. DNA-ring with Cy5 modification

All time and temperature studies were performed on DNA-rings, in which all oligonucleotides were modified with Cy5 at the 5'-terminus end.

4.1.4.1. Time studies

The time studies for Cy5 were performed in the same way as in the Cy3 case i.e. with a heating block (QBH2 Grant) where samples were put and taken out at different times with a 20 min interval, while the temperature was held constant at 50 °C.

As seen in fig 4.8, both monomer and hexagon bands are formed while it is unclear if any 12 mer bands exist. Many of the samples seem to have given rise to bands at the wells of the gel, which is due to that the samples have stuck there.

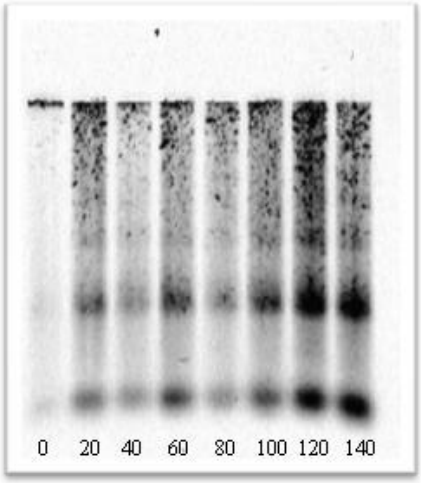


Fig 4.8. Gel image from time experiments with each lane labeled with respective time in min. (From left: 0 to 140 min).

Fig 4.9. Collected intensities for the time studies of DNA-rings with Cy5.

As seen in fig 4.9 no rings larger than the 6 mer were formed. The monomer yield seems to be the same (around 44%) except at 120 min, where it seems to be a peak for the monomer (83%). The hexagon formation is around 55% at all times.

4.1.4.2. Temperature studies

The hybridization was performed in the same way as for Cy3, but with heating block QBH2 Grant. As seen in fig 4.10, DNA-rings up to the 24 mer ring were formed. The sample at 30 °C was lost when loading the gel and therefore no bands could be detected for this temperature on the gel, also it seems that most of the samples that were loaded have stuck in the well.

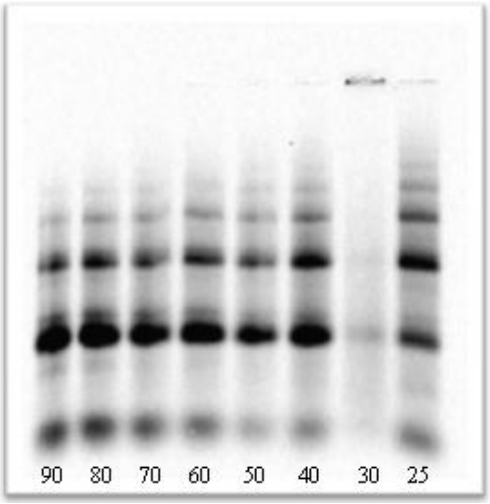


Fig 4.10. Gel image of Cy5 temperature studies.
(From left: 90 to 25 °C).

In figure 4.11, the intensities for each band type are presented as well as the sum of intensities for the 12 mer, 18 mer and 24 mer. The sum of these shows a collected intensity for the larger ring formation which is compared to the monomer and hexagon formation (fig 4.12).

It is observed in fig 4.11, that the formation of the monomer and hexagon decreases the lower temperature while the formation of 12 mer, 18 mer and 24 mer increases with decreasing temperature.

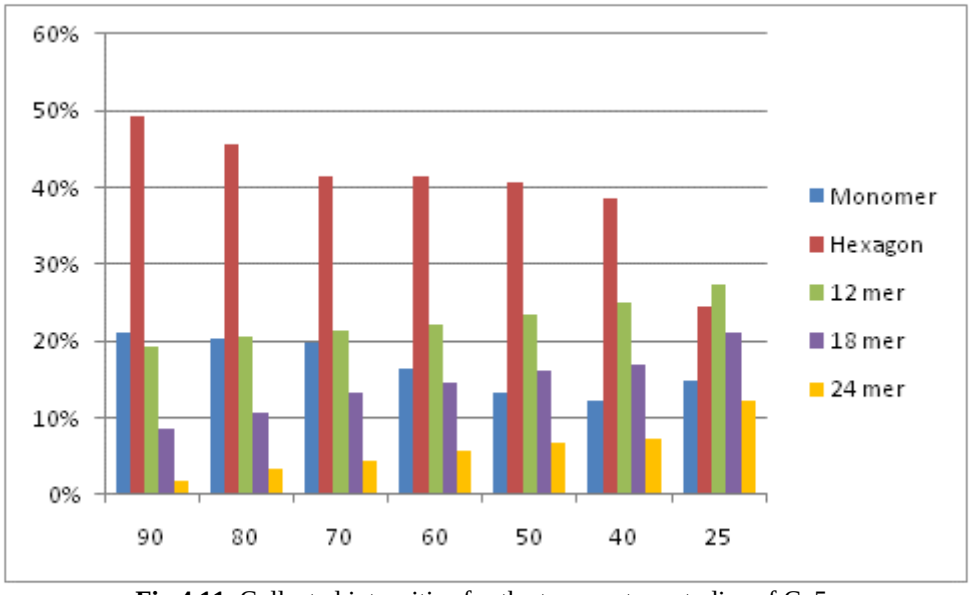


Fig 4.11. Collected intensities for the temperature studies of Cy5.

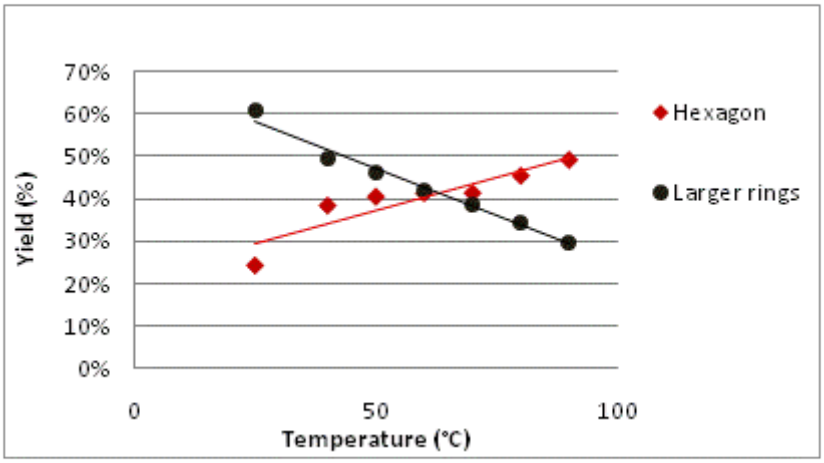


Fig 4.12. Collected intensities for hexagon and larger rings.

Table 5 shows the linear regression and the R^2 value for each band type. The linear regression and fig 4.12, indicates that the formation of larger DNA-rings decreases the higher temperature. When the formation of larger constructs increases it is also seen that the monomer and hexagon formation decreases. Thus larger rings form at the expense of the hexagon formation.

Table 5. Linear regression for each band type.

Band type	**Linear regression**	**R^2**
Monomer	y = -0,0014x +0,0874	0,745
Hexagon	y = 0,0031x+0,2165	0,829
12 mer	y = -0,0012x+0,2998	0,978
18 mer	y = -0,0018x+0,2519	0,984
24 mer	y = -0,014x + 0,1445	0,942
Larger rings	y = -0,0045x +0,696	0,979

4.2. Linked DNA-ring

Since the other part of the thesis project was to study the formation of linked DNA-rings, the following results are focused on this part.

To prove that linked DNA-rings are formed, a ladder with increasing number of oligonucleotides was performed on the linked DNA-ring. A Cy5-modified oligonucleotide was used to visualize the different structures.

Table 6. Samples for ladder of linked DNA-ring.

Sample	Oligonucleotides	Additional information
1.	$H5^{Cy5}$	1 mer
2.	$H5^{Cy5}$, H2	2 mer
3.	$H5^{Cy5}$, H2, Link 2	3 mer
4.	$H5^{Cy5}$, H2, Link 2, H3	4 mer
5.	$H5^{Cy5}$, H2, Link 2, H3, H4	5 mer
6.	$H5^{Cy5}$, H2, Link 2, H3, H4, Link 1	6 mer (Linked DNA-ring)
7.	$H5^{Cy5}$, H2, Link 2, H3, H4, Link 1, 1', 6'	10 mer DNA-ring
8.	$H5^{Cy5}$, H2, H6, H3, H4, H1	DNA-ring without link

Fig 4.13. Gel image of ladder. (From left: sample 1 to 8).

In fig 4.13, it is observed that a band migrates shorter distance the more oligonucleotides added, thus indicating that larger constructs are formed. Also, band 4 and 5 are very weak, which can be explained with problems regarding the H3 oligonucleotide, seen in previous experiments (see appendix). Sample 6 and 7 are very similar suggesting that the 10 mer DNA-ring and the linked DNA-ring have similar formations.

Intensity measurements of sample 6 to 8 showed that the formation of 6 mer was almost identical for the linked DNA-ring and the 10 mer DNA-ring (see fig 4.14).

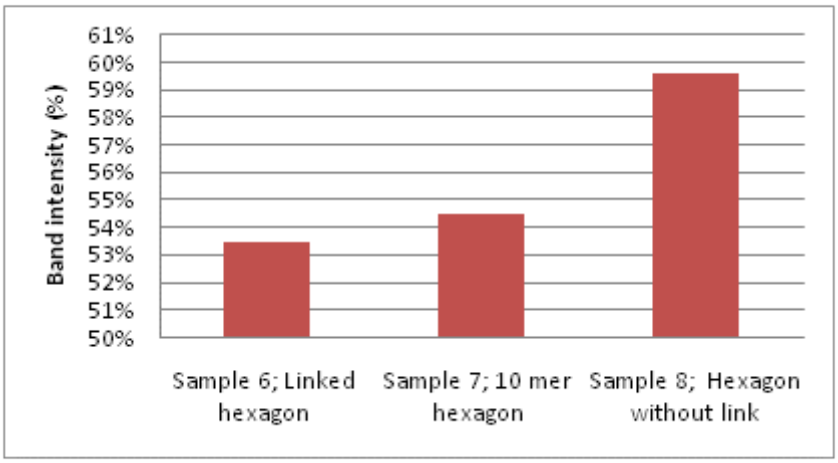


Fig 4.14. Intensities for the hexagonal and the biphenyl band (sample 6 to 8).

Since the linked DNA and the 10 mer DNA had similar formations, the future experiments were focused on the linked DNA.

Because of the problems with H3 shown in fig 4.13 and the fact that the DNA-ring can be built from the H3 oligonucleotide instead of the H5, it was desirable to study the build up from this starting point. Therefore, a reverse ladder (compared to above) was designed according to table 7.

Table 7. Sample information of reverse ladder.

Sample	Oligonucleotides	Additional information
1.	$H3^{Cy5}$	1 mer
2.	$H3^{Cy5}$,H4	2 mer
3.	$H3^{Cy5}$,H4,Link 1	3 mer
4.	$H3^{Cy5}$,H4,Link 1, H5	4 mer
5.	$H3^{Cy5}$,H4,Link 1, H5, H2	5 mer
6.	$H3^{Cy5}$,H4,Link 1, H5, H2, Link 2	6 mer (Linked DNA-ring)

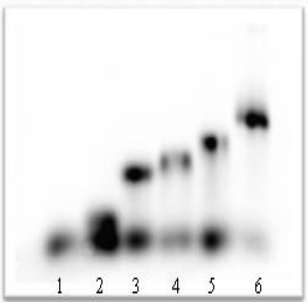


Fig 4.15. Gel image for reverse ladder. (From left: sample 1 to 6).

As seen in fig 4.15, the build up from the H3 oligonucleotide is quite similar to the H5, showing almost all structures, indicating that one can build the linked DNA-ring by using H3 or H5 as starting point.

4.2.1. Hybridization

Since this system is a completely new system, it was desirable to know more about the formation of the system and which hybridization that was best for this system. Therefore, different hybridization techniques were tested for the linked DNA-ring to see which one gave the best effect on the formation. Table 8 presents the different samples and which hybridization that was used. In this experiment, Cy5 was used to visualize the samples.

In table 8 below, the yield of the hexagonal structure is presented for the different hybridization techniques.

Table 8. Collected intensities for each hybridization technique.

Sample	Hybridization	Yield of hexagon (%)
1.	Heat to 90 °C for 5 min	30
2.	Heat to 90 °C for 5 min, cool down to 50 °C	35
3.	Heat to 90 °C for 5 min, cool down to room temperature, heating block QBT1 Grant	52
4.	Heat to 90 °C for 5 min, cool down to room temperature, heating block QBH2 Grant	20
5.	Mix in room temperature, day before gel electrophoresis	29
6.	Mix in room temperature, same day as gel electrophoresis	5
7.	Heat to 85 °C, cool down to 5,5 °C over three hours, with a linear gradient.	70
8. Reference sample, DNA-ring without linkers	Heat to 85 °C, cool down to 5,5 °C over three hours, with a linear gradient.	52

The results showed that the best hybridization for the hexagon was the computer assisted hybridization, in which the sample was heated to 85 °C and then cooled down to 5,5 °C over three hours, with a linear gradient (sample 7), rendering a yield of 70% (see table 8).

Fig 4.16. Gel image for the hybridization experiment for the linked DNA-ring.
(From left: sample 1 to 8).

Also, as seen in fig 4.16, sample 2 and 4 have formed what could be larger rings while the other samples show only the hexagonal band as the major product. Most of the samples also show that constructs have formed poorly since it exists bands below the hexagonal band, which indicates that byproducts e.g. monomer, dimer and trimer have formed. However, apart from sample 6, the hexagon is the major product in all samples.

4.2.2. Formation of linked DNA-ring

Since linked DNA-rings can be connected to each other with the help of the Glue oligonucleotide, experiments were performed in an attempt to form the biphenyl structure as well as to polymerize the linked DNA-rings. The polymerization can be carried out in two ways, as mentioned in chapter 3.2.1.

4.2.2.1. Polymerization methods

In this experiment, the Glue was added to samples containing DNA-rings with both links and samples containing mixtures of DNA-ring with Link 1 and DNA-ring with Link 2, before and after hybridization (see table 9). Sample 5-8 were used as reference samples in the experiments. Sample 5 and 6 contained an equimolar mixture of the DNA-ring with Link 1 and DNA-ring with Link 2, while sample 7 and 8 only contained the DNA-ring with Link 1 respectively the DNA-ring with Link 2.

Table 9. Sample information.

Sample	Oligonucleotides	Additional information
1.	$H5^{Cy5}$, H2, Link 2, H3, H4,Link 1,Glue	DNA-ring with both links Glue added before hybridization
2.	$H5^{Cy5}$, H2, Link 2, H3, H4, Link 1, Glue	As sample 1
3.	$H5^{Cy5}$,'H2, Link 2, H3, H4, Link 1, Glue	DNA-ring with Link 1 + DNA-ring with Link 2 mixed with Glue before hybridization
4.	$H5^{Cy5}$, H2, Link 2, H3, H4, Link 1, Glue	As sample 3
5.	$H5^{Cy5}$, H2, Link 2, H3, H4, Link 1	DNA-ring with Link 1 + DNA-ring with Link 2 mixed after hybridization
6.	$H5^{Cy5}$,'H2, Link 2, H3, H4,Link 1	As sample 5
7.	$H5^{Cy5}$,'H2, H6, H3, H4, Link 1	DNA-ring with Link 1
8.	$H5^{Cy5}$,'H2, Link 2, H3, H4, H1	DNA-ring with Link 2

For sample 1-4 it was observed that hexagonal and biphenyl DNA had formed. Intensities were measured for sample 1-4, to see which gave the best result in terms of formation.

Table 10. Band intensities.

Sample	Hexagon	Biphenyl
1.	78 %	22 %
2.	78 %	22 %
3.	48 %	52 %
4.	48 %	52 %

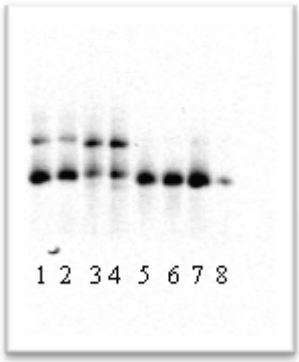


Fig 4.17. Gel image from polymerization experiments . (From left: sample 1 to 2).

It is observed that the formation of biphenyl DNA is about 50% for sample 3 and sample 4. It is surprising that no bands corresponding to longer hexagonal polymers than two are present in sample 1 and 2, indicating that DNA-rings that contain both links don't seem to polymerize well. As expected, polymerization was not seen when no Glue was added (sample 5-8).

4.2.2.2 Hybridization time of Glue

Since no clear polymerization is visible, it was desirable to study more thoroughly if more than two hexagons of linked DNA-rings could be formed. If the kinetics are slow, then the hybridization time of the reaction of Glue is important. The reaction time after addition of Glue was therefore varied to observe how this affected the polymerization. In this experiment the DNA-ring with both links and an equimolar ratio of Glue was studied.

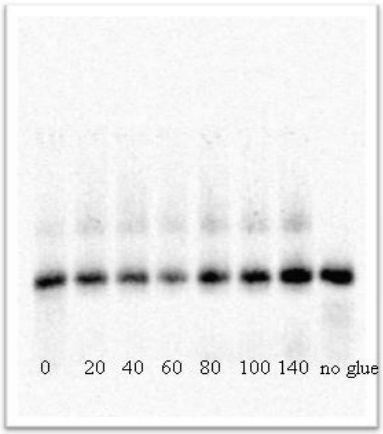


Fig 4.18. Hybridization time of Glue with each lane labeled with time of Glue. (From left: 0-140 min, last sample: linked DNA-ring with no Glue).

As seen in figure 4.19, no monomer is present which gives rise to high formation of the hexagon and the biphenyl DNA.

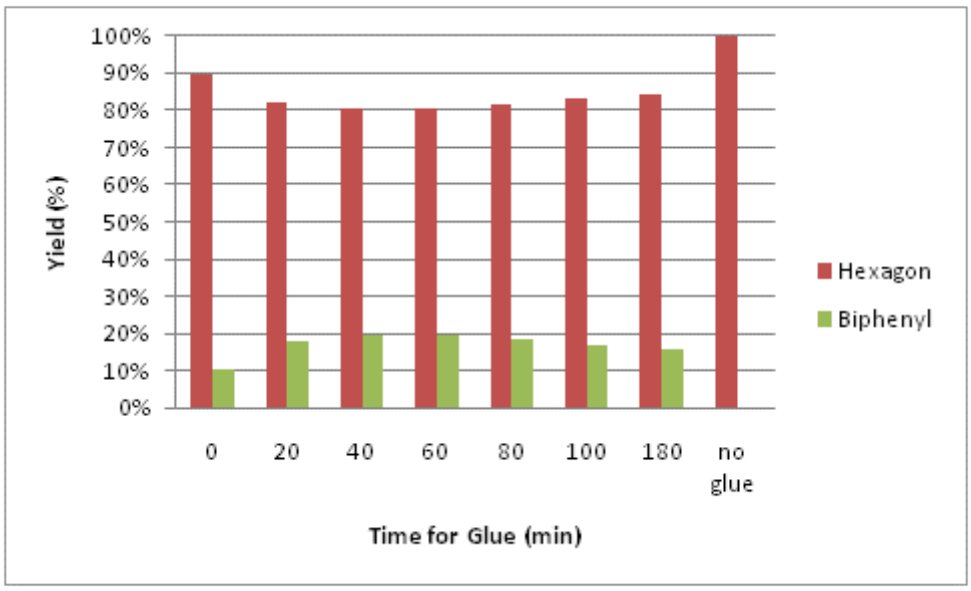


Fig 4.19. Intensities for addition of Glue at different times.

The hexagon formation is high while the biphenyl formation is low, thus indicating that polymerization of linked DNA-rings is low. No bands are observed for DNA-rings larger than the biphenyl, thus proposing that no more than two linked of DNA-rings can be connected. It could also be that the amount of Glue compared to the amount of the volume of the protruding arms of the linked DNA-ring affects the polymerization, if the stoichiometry of Glue is too large it is possible that it quenches the reaction by hindering DNA-rings to connect.

4.2.2.3. Ratio of Glue

Since the stoichiometry of Glue to protruding arms and oligonucleotide could be the reason of the inefficient polymerization, the amount of Glue was varied. It was also tested to add Glue before and after hybridization of the DNA, to observe if this had any effect on the polymerization. In this experiment, the linked DNA ring with both links was used to see if any polymerization beyond the two linked DNA-ring could be performed. The samples used were prepared according to table 11.

Table 11. Sample information.

Sample	**Oligonucleotides**	**Ratio of Glue to protruding arms**	**Additional information**
1.	$H5^{Cy5}$, H2, Link 2, H3, H4, Link 1, Glue	1	Before hybridization
2.	$H5^{Cy5}$, H2, Link 2, H3, H4, Link 1, Glue	1/2	
3.	$H5^{Cy5}$, H2, Link 2 , H3, H4, Link 1, Glue	1/3	
4.	$H5^{Cy5}$, H2, Link 2, H3, H4, Link 1, Glue	1/6	
5.	$H5^{Cy5}$, H2, Link 2, H3, H4, Link 1, Glue	1	After hybridization
6.	$H5^{Cy5}$, H2, Link 2, H3, H4, Link 1, Glue	1/2	
7.	$H5^{Cy5}$, H2, Link 2, H3, H4, Link 1, Glue	1/3	
8.	$H5^{Cy5}$, H2, Link 2, H3, H4, Link 1, Glue	1/6	

It can be observed in fig 4.20 that sample 1 and 6 formed hexagon and biphenyl while sample 7 and 8 only formed hexagon. No three ring linking was observed, thus indicating that polymerization beyond two rings is either very low or nonexistent.

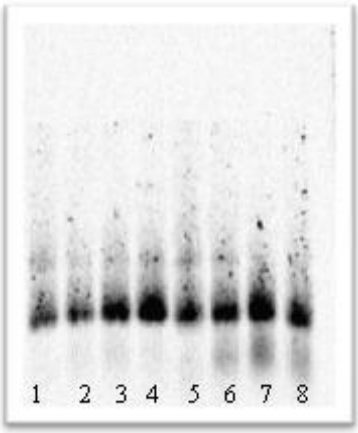


Fig 4.20. Gel image for ratio of Glue
(From left Sample 1 to 8).

Fig 4.21 and 4.22 show that the hexagon formation increases the lower Glue ratio while the biphenyl formation seems to decrease the lower Glue ratio.

Fig 4.21. Intensities for addition of Glue before hybridization.

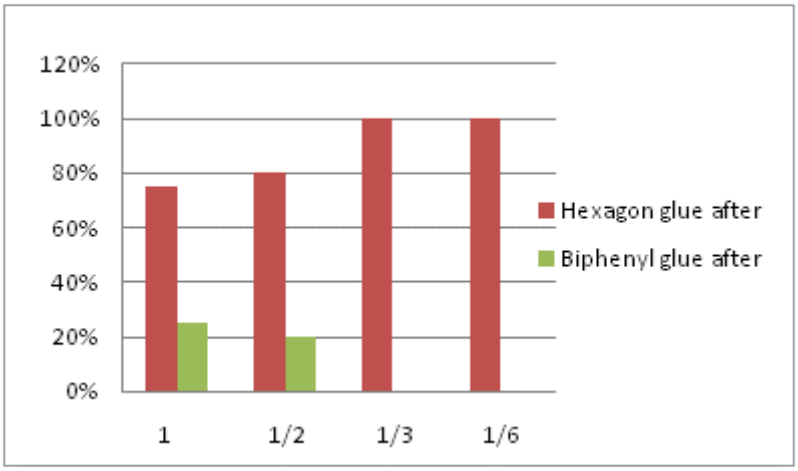


Fig4.22..Intensities for addition of Glue after hybridization.

As seen in fig 4.21 and 4.22 less hexagon is formed when Glue is added before hybridization in comparison to when it is added after hybridization. The results also indicate that the less hexagon formation the more biphenyl formation.

4.3. Melting curves

This part will present how the melting temperature depends on DNA-ring type. The different types that will be studied is the DNA-ring with and without cyanine and the linked DNA-ring with and without Glue.

4.3.1. Comparing DNA-ring with linked DNA-ring

Since it is important to observe if the unmodified DNA-ring and the unmodified linked DNA-ring differ in stability, following results will deal with comparing the melting curves for each of these cases. The increase in absorption for the heating step is most likely an instrumental error, and doesn't affect the melting temperature and is seen in every experiment.

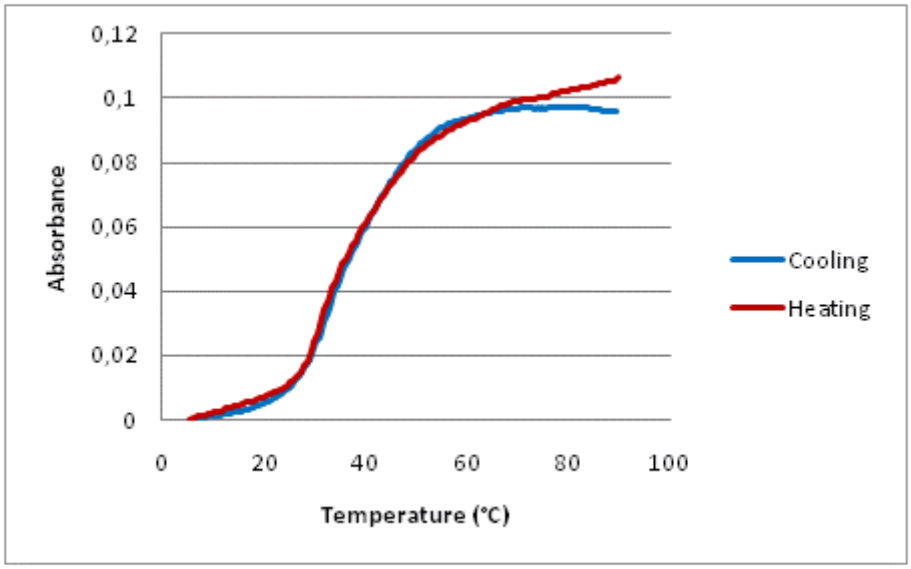


Fig 4.23. Melting curve for the DNA-ring with all six oligonucleotides unmodified.

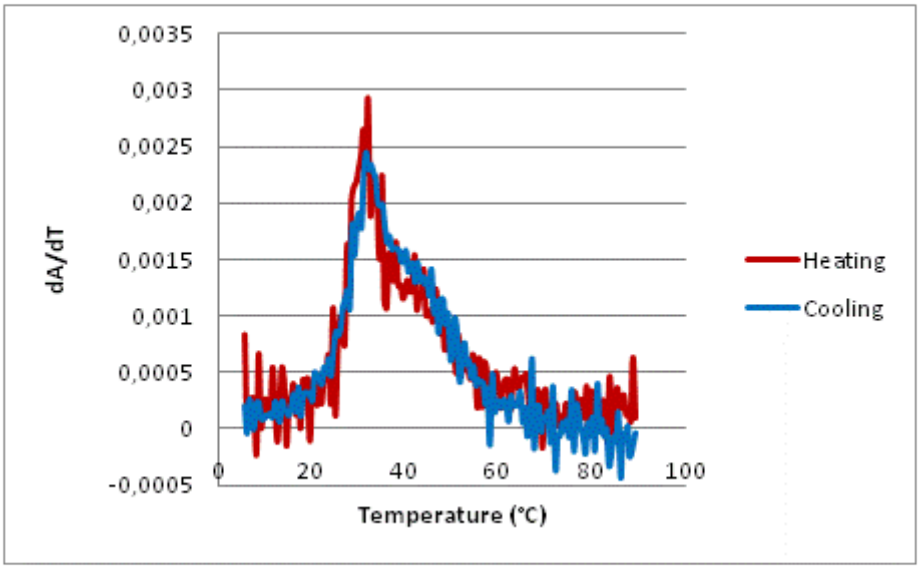


Fig 4.24. Derivative of melting curve for the DNA-ring with all six oligonucleotides unmodified.

From these results it was calculated that the DNA-ring has a melting temperature of 32 °C well agreement with previous results.[3] Also, it can be seen that the derivate decreases with cooling, comparing to the heating, but both still have similar appearance.

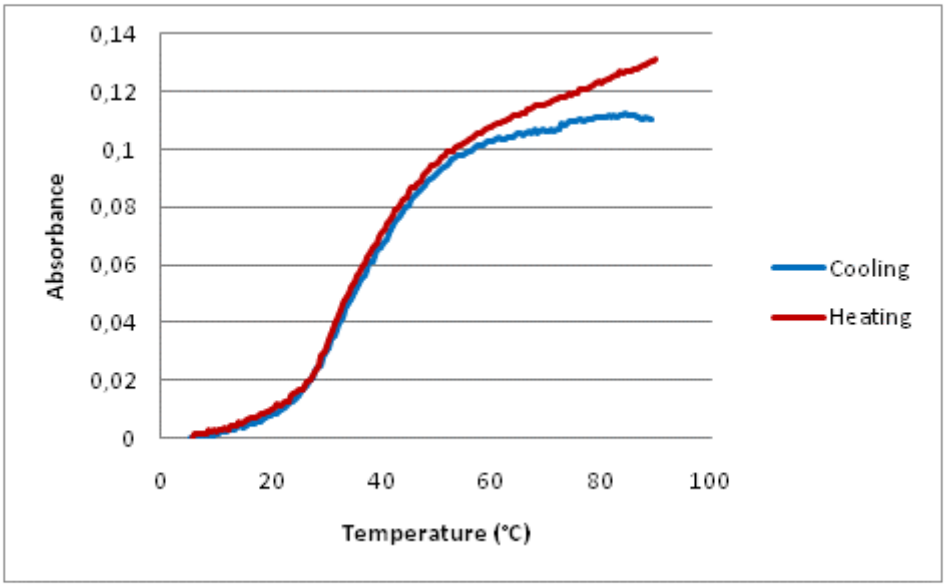


Fig 4.25. Melting curve for linked DNA-ring with all six oligonucleotides unmodified.

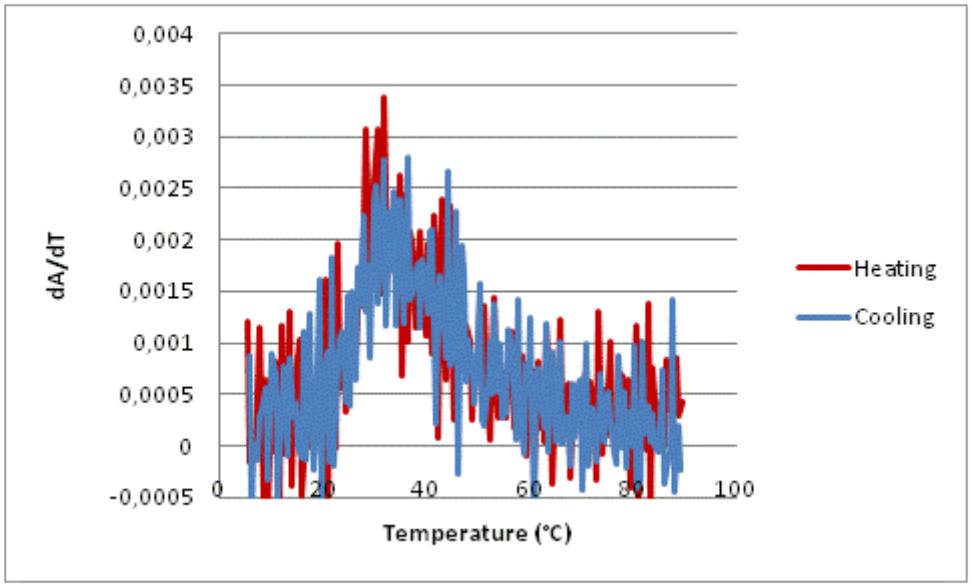


Fig 4.26. Derivative of melting curve for linked DNA-ring with all six oligonucleotides unmodified.

The melting temperature of the linked DNA-ring was calculated to 32 °C. According to these results, the linked DNA-rings have the same melting temperature as the unlinked DNA-rings thus indicating that the melting temperature is unaffected by adding links to the DNA-rings.

4.3.2. With and without Glue

It is important to know if Glue changes the melting temperature of the linked DNA-ring, since this can tell something about the linked DNA-ring stability with and without Glue. Fig 4.27 and 4.28 shows the melting curve and its derivate for the linked DNA-ring with Glue, which is compared to fig 4.25 and 4.26 (DNA-ring).

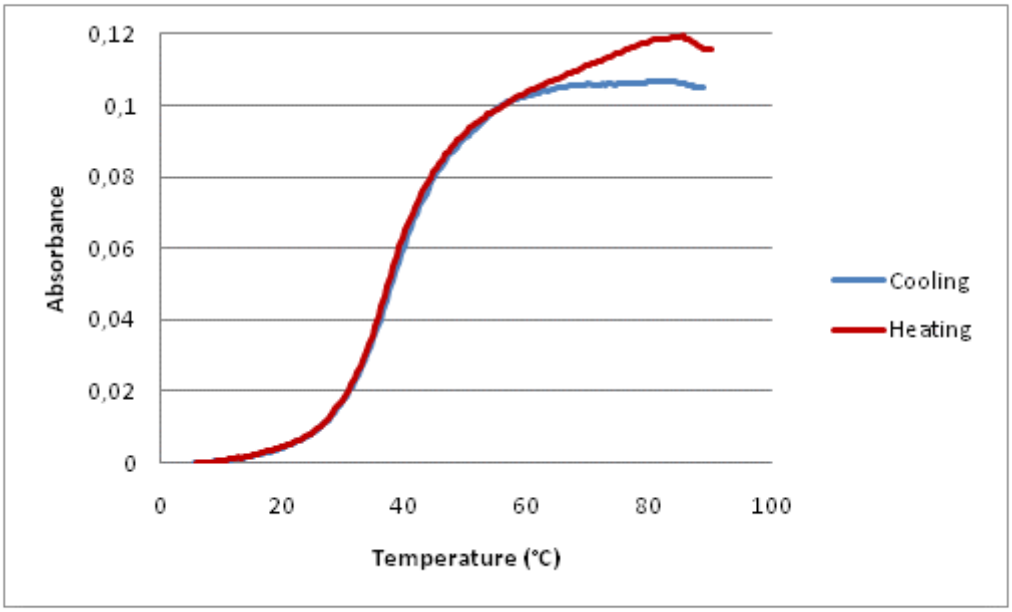


Fig 4.27. Melting curve for the linked DNA-ring with Glue and all six oligonucleotides unmodified.

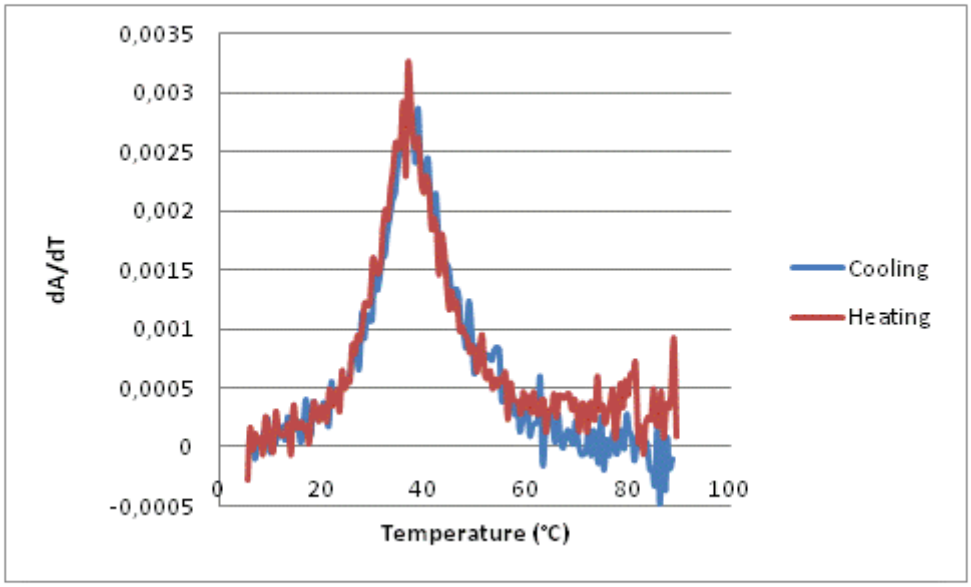


Fig 4.28. Derivative of melting curve for the linked DNA-ring with Glue and all six oligonucleotides unmodified.

This melting curve gave a melting temperature of 38 °C, thus the Glue oligonucleotide has increased the melting temperature of the linked DNA-ring from 32 °C to 38 °C. As seen in fig 4.28, no big difference between the heating and the cooling step exists for the linked DNA-ring with Glue.

4.3.3. Cy3 effect

Since one major part of this project was to study the Cy3 and Cy5 effect, melting curves were established for the DNA-ring with Cy5 and Cy3 modifications. This part will show the Cy3 effect on the DNA-ring by comparing the melting curves for the DNA-rings with and without Cy3.

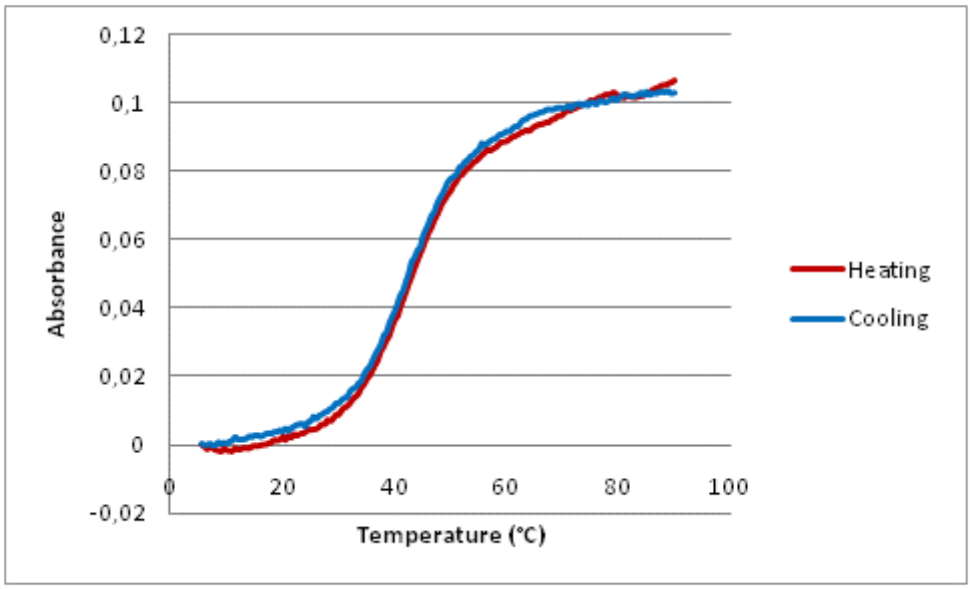


Fig 4.29. Melting curve for the DNA-ring with all six oligonucleotides Cy3 modified.

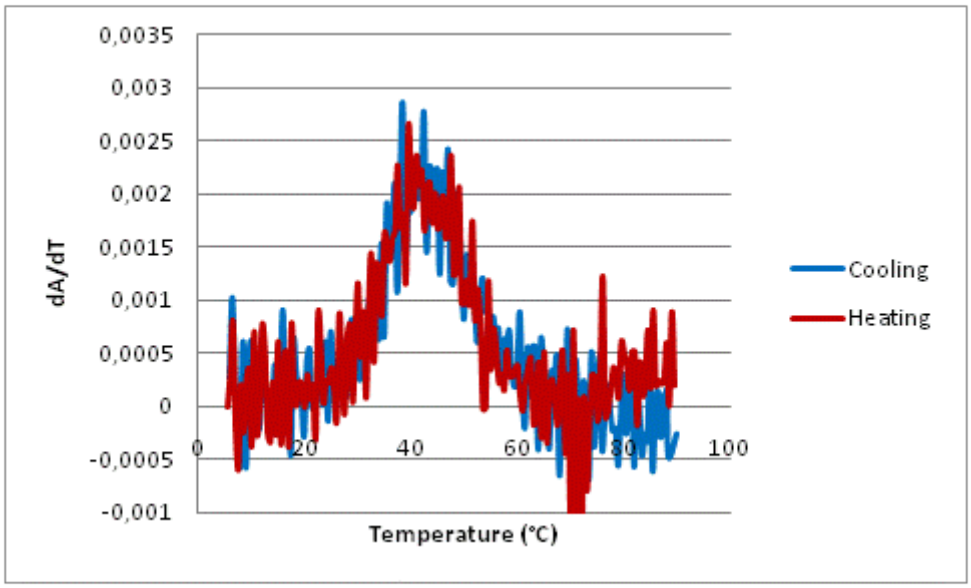


Fig 4.30. Derivative of melting curve for the DNA-ring with all six oligonucleotides Cy3 modified.

These results yielded a melting temperature of 43 °C, which is significantly larger than the melting temperature for the unmodified DNA-ring. The reason behind this is probably due to that the fluorophore stabilizes the construct which could have some effect on the formation of larger rings.[15]

4.3.4. Cy5 effect

This part will focus on the Cy5 effect by comparing DNA-ring with and without Cy5 modification i.e. comparing fig 4.25 and 4.26 with the figures below.

Since it was interesting to see if the melting temperature of the DNA-ring increased the more fluorophore, experiments were performed with increasing number of Cy5 fluorophores in the DNA-ring. The numbers of Cy5 that were tested was: 1 Cy5, 3 Cy5, 5 Cy5 and 6 Cy5. The oligonucleotide samples used can be seen in table 12.

Table 12. Samples for melting curves.

Sample	**Unmodified oligonucleotides**	**Cy5 modified oligonucleotides**
1 Cy	H3	H4, H1, H5, H2, H6
3 Cy	H3, H4, H1	H5, H2, H6
5 Cy	H3, H4, H1, H5, H2	H6
6 Cy	H3, H4, H1, H5, H2, H6	

4.3.4.1. DNA-ring with one Cy5

Fig 4.31 presents a similar melting cure for both heating and cooling, which is also seen in the derivate (fig 4.32).

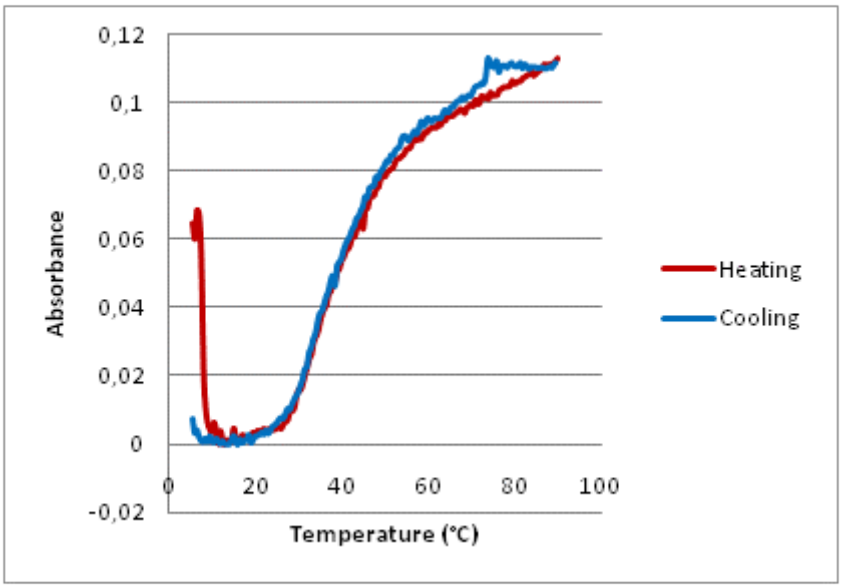


Fig 4.31. Melting curve for DNA-ring with one Cy5.

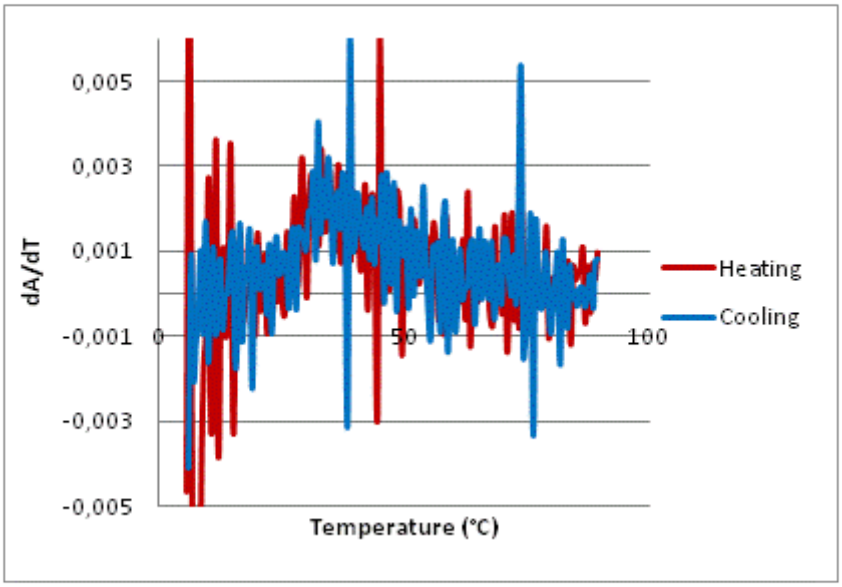


Fig 4.32. Derivative of melting curve for DNA-ring with one Cy5.

When comparing these curves with the unmodified DNA-ring (fig 4.23 and 4.24), it is seen that the DNA-ring with one Cy5 has a higher melting temperature than the unmodified DNA-ring. The DNA-ring with one Cy5 has T_m= 35 °C while the unmodified DNA-ring has T_m= 32 °C. This indicated that the Cy5 fluorophore stabilizes the DNA-ring, just as the Cy3 fluorophore.

4.3.4.2. DNA-ring with three Cy5

As seen in fig 4.33 and 4.34, the melting temperature was higher for this case compared to the
DNA-ring with one Cy5. The DNA-ring with three Cy5 had the melting temperature 39 °C, while the DNA-ring with one Cy5 had 35 °C.

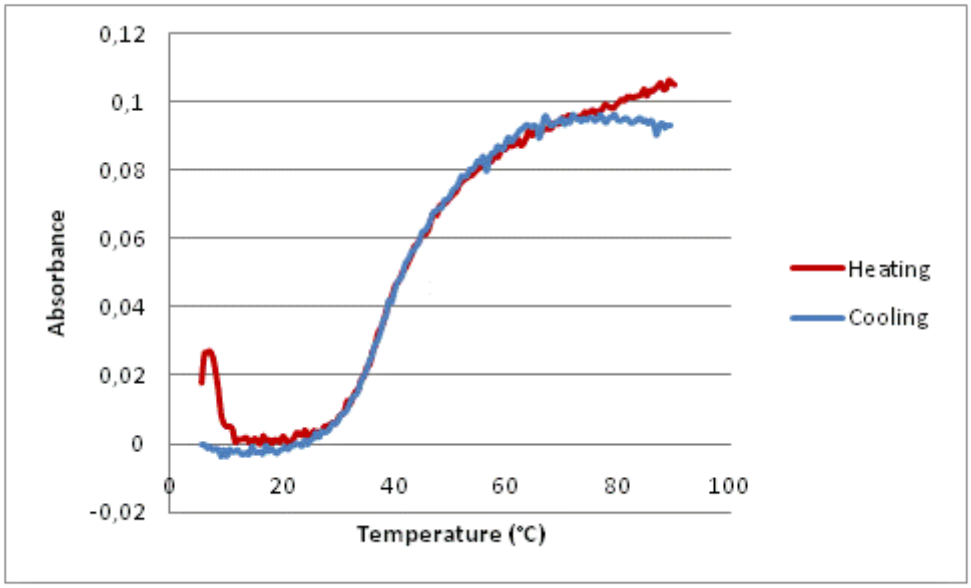


Fig 4.33. Melting curve for DNA-ring with three Cy5.

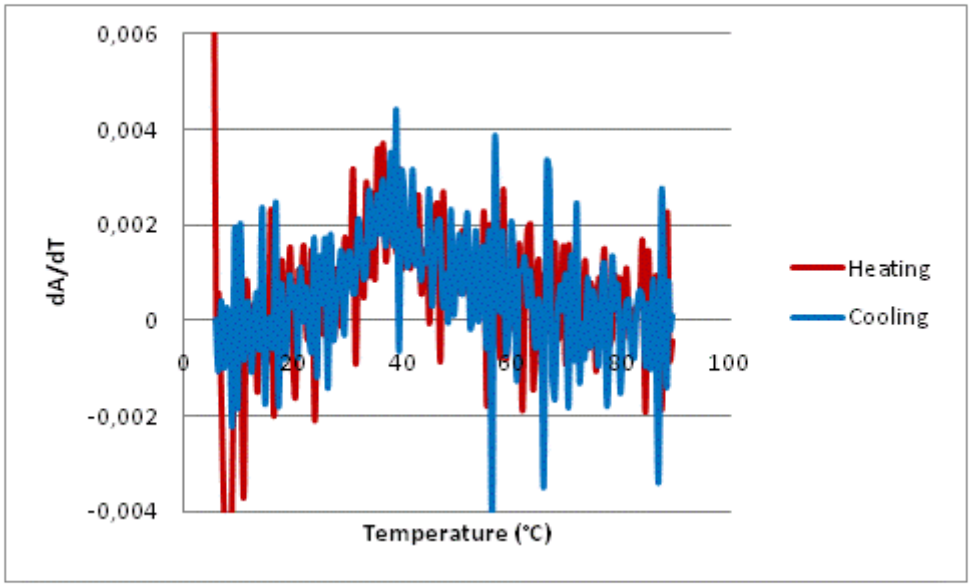


Fig 4.34. Derivative of melting curve for DNA-ring with three Cy5.

Since these results showed a higher melting temperature for the DNA-ring with three Cy5 compared to the DNA-ring with one Cy5 and the unmodified DNA-ring, this indicates that the more fluorophore the higher the melting temperature.

4.3.4.3. DNA-ring with five Cy5

This experiment showed a melting temperature of approximately 41 °C, which verifies the fact that the melting temperature increases the more fluorophore. The big jump at about 25-30 °C for the cooling step is most likely explained by an instrumental error.

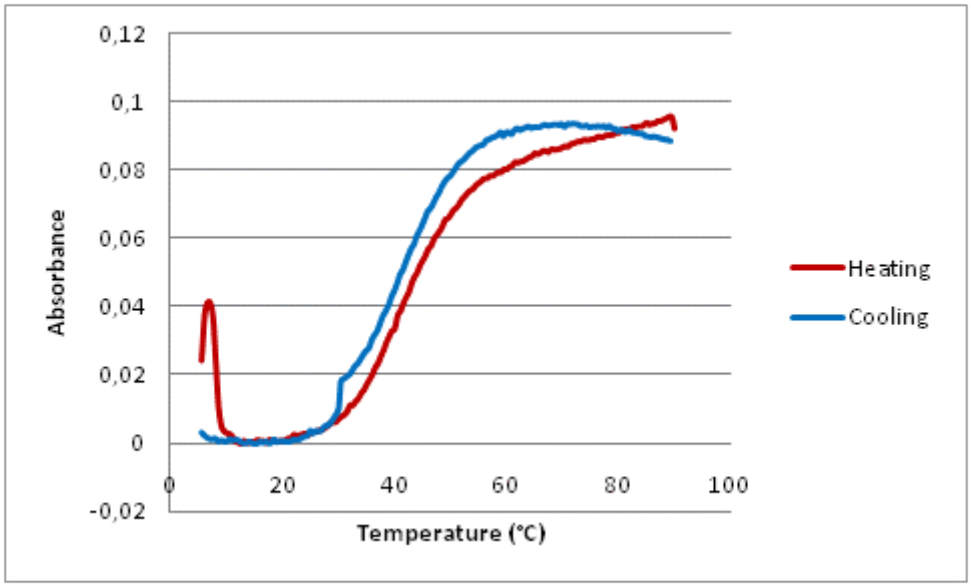


Fig 4.35. Melting curve for DNA-ring with five Cy5.

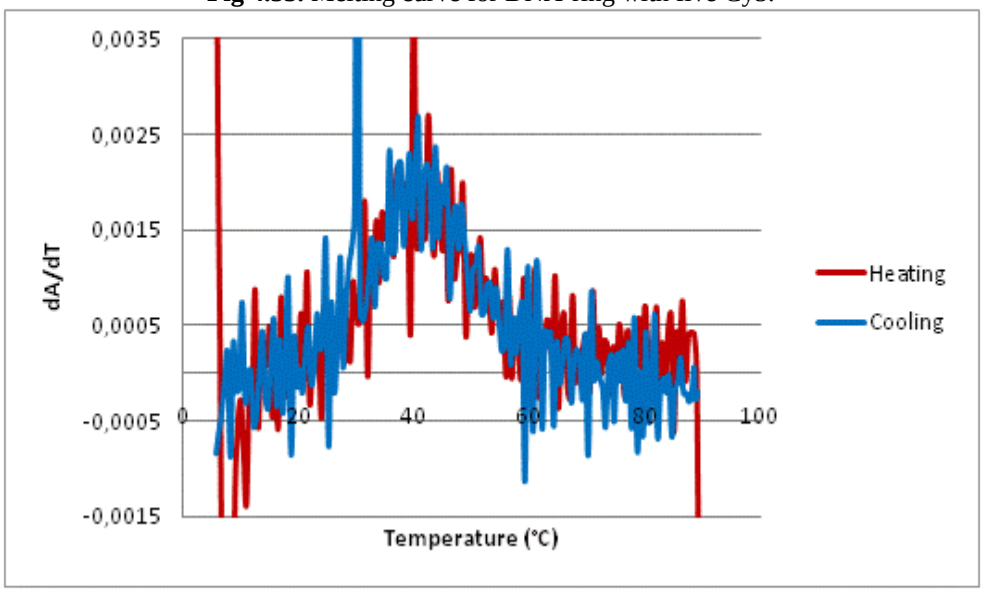


Fig 4.36. Derivative of melting curve for DNA-ring with five Cy5.

4.3.4.4. DNA-ring with six Cy5

The melting temperature was 42 °C and this indicates that the DNA-ring with six Cy5 is more stable than the unmodified DNA-rings, which is due to that fluorophores can stabilize the DNA duplex.[15] If comparing to the Cy3 case, is it observed that Cy3 and Cy5 have more or less the same effect on the melting temperature.

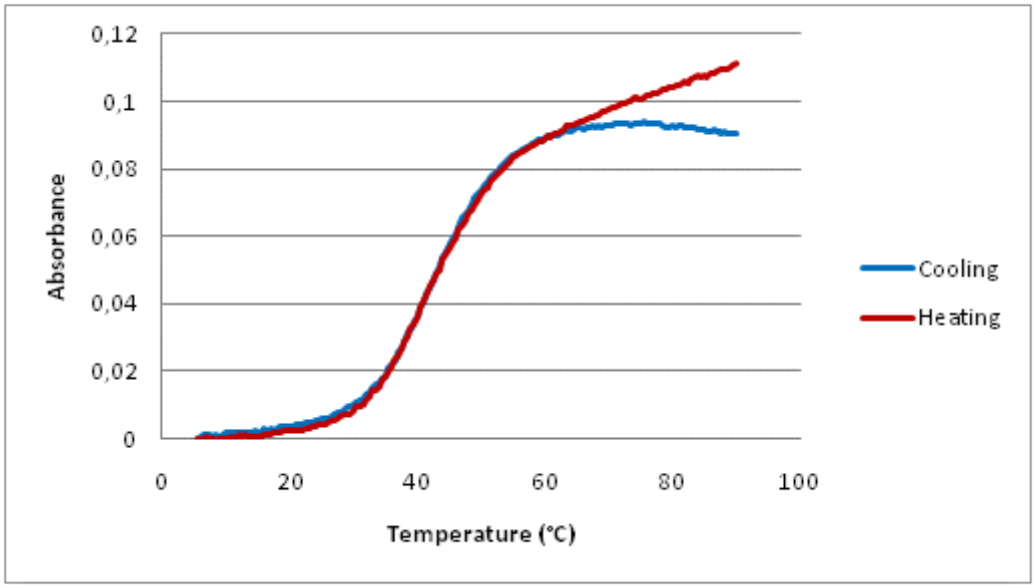


Fig 4.37. Melting curve for the DNA-ring with all six oligonucleotides Cy5 modified.

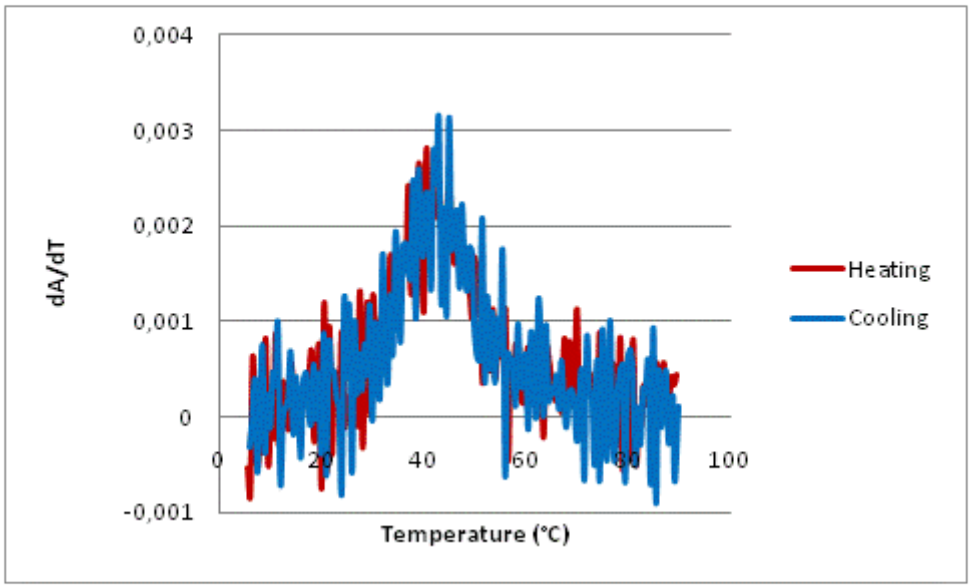


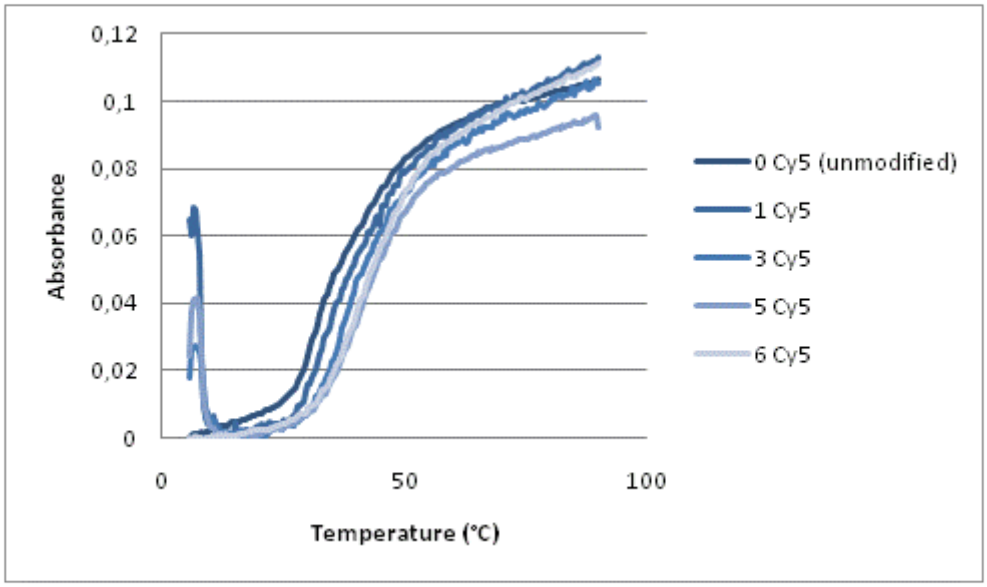
Fig 4.38. Derivative of melting curve for the DNA-ring with all six oligonucleotides Cy5 modified.

As seen in table 13, the melting temperature increases the more fluorophore, which indicates that the more fluorophore the more stabilizing of the DNA-ring.

Table 13. Summary for all melting temperature for the Cy5 case.

DNA-ring with:	**T_m (°C)**
0 Cy5 (unmodified)	32
1 Cy5	35
3 Cy5	39
5 Cy5	41
6 Cy5	42

The fact that the melting temperature increases the more fluorophore can also easily been seen in fig 4.39 that shows each melting curve (heating step) for the DNA-ring with increasing number of oligonucleotides modified.



4.39. Melting curves for DNA-ring with increasing number of oligonucleotides.

5. Conclusions

5.1. DNA-ring with cyanine modification

By constructing a ladder of increasing number of oligonucleotides it has been verified that DNA-rings are formed, and that 12 mer rings can be formed. The ladder with increasing number of Cy3 has shown that larger ring formation may be enhanced by increasing number of fluorophore.

The results from the temperature experiments with Cy5 and Cy3 modified DNA, has indicated that larger DNA-rings forms better the less monomer. Especially the results from the Cy5 experiments have shown that, the less formation of smaller rings (6 mer and monomer), the larger formation of 12 mer and 18 mer. It has also been shown that a lower temperature results in more formation of the 12 mer and 18 mer ring. Also, it has been verified in temperature studies of Cy5 that larger rings form on the expense of smaller rings.

The melting curves have shown that fluorophores increase the melting temperature which is due to that fluorophores stabilize DNA.[15] Thus the formation of DNA-rings is effected by the fluorophore.

The time experiments have shown that the formation of Cy3 and Cy5 modified DNA-rings seems to have a constant formation at 50 °C for the monomer, hexagon and 12 mer at all times. Results have also indicated that the DNA-ring system is sensitive to high formation of hexagon and high yield of monomer.

5.2. Linked DNA-ring

Results have shown that DNA-rings with protruding arms (links) can be formed and that the biphenyl DNA is formed quite effectively. It is unclear if polymerization of linked DNA-rings is possible. Also, it has been proposed that the 10 mer DNA-ring formed in similar fashion as the linked DNA-ring. Also it has been shown that the linked DNA-ring can be built up from both the H5 and H3 oligonucleotides.

Hybridization of the linked DNA-ring is best when using a computer assisted water bath with a linear gradient. The rest of the hybridization strategies that have been tested have shown formation of byproducts which are not desirable

It has been indicated that polymerization of DNA-rings with both links can be possible. Although the polymerization has shown to be quite ineffective for the DNA-rings containing Link 1 and Link 2. Also, the linked DNA-rings seem to have the same melting temperature as the unlinked DNA-rings thus indicating that the melting temperature is unaffected by linking of DNA-rings.

It has also been shown that the Glue oligonucleotide is most effective before hybridization of the DNA-rings. The Glue seems to connect two linked DNA-rings but it is unclear if it can polymerize more rings. The stoichiometry of Glue is clearly a factor that effects the polymerization in some way.

6. Future work

It is desirable to perform more experiments with the Glue oligonucleotide to see if the polymerization can be more effective. The kinetics of the reaction could be something to investigate further. For example one can do more experiments by adding Glue at different times to see whether Glue needs more time to connect the linked DNA-rings. Also it is interesting to see whether the Glue oligonucleotide needs several days to polymerize the DNA, so therefore one could do a long time experiment with Glue.

It is also desirable to do more time experiments with the Cy3 and Cy5 modified DNA-rings, so that more temperatures of the time experiments are studied, for example one could test 40 °C and 30 °C that lie below the melting temperatures of the different DNA-rings.

Another alternative is to study the DNA-rings with a different setup of the gel electrophoresis since the phosphate buffer that was used had quite high ion strength and therefore resulted in a quite high current in the gel. The buffer could be changed to a phosphate buffer with lower Na^+ concentration or where the sodium is replaced with magnesium.

Mobility studies of the DNA-rings are also desirable since these can show how the DNA-rings move in the gel depending on the gel concentration. By varying the gel concentration and the voltage, one could deduce both the mobility and charge of the DNA-rings.

Atomic force microscopy is another technique that should be tested for the DNA-rings, especially the linked DNA-rings, so one can study and visualize them.

Click fixation chemistry is another route of studying the DNA-rings. By using this method that incorporate C-X-C links, one can use click chemistry to e.g. attach fluorophores on DNA and incorporate chemical cross linking on DNA. By doing this, the DNA-rings can be used to for building larger networks that are functionalized and fixated. [18]

7. Acknowledgements

My examiner Bengt Nordén

Especially thanks to my supervisor Erik Lundberg. Thanks for all your help, good support and all interesting discussions.

Thank you Marcus Wilhemsson for helping me with a project title, and thank you Karl Börjesson for lending me a cyvette holder for the melting curve experiments.

Björn Åkerman for discussions and help about gel electrophoresis.

Calin Plesa for interesting discussions about our thesis projects about DNA-rings and gel electrophoresis.

Thanks Susanne Ryberg for being my opponent and thank you Bobo Feng for proof reading my report.

A big thank to all my friends at the bachelor programs of Bioengineering, Chemistry with Physics, Chemistry, Engineering mathematics and Physics for all support and fun times at Chalmers. Also thanks to all my friends and family outside Chalmers.

Finally, thanks to all the employees and master thesis students at Physical Chemistry that welcomed me and helped me.

References

1. Bath, J & Tuberfield, A; (2007); DNA nanomachines; *Nature nanotechnology*, 104.
2. Sandin, P; Tumpane, J; Börjesson, K; Wilhelmsson, L.M; Brown, T; Nordén, B; Albinsson, B & Lincoln, P; (2009), Thermodynamic aspects of DNA nanoconstruct stability and design, *Journal of Physical Chemistry, 113*,5941-5946.
3. Tumpane, J; Sandin, P; Kumar, R; Powers,V, E.C; Lundberg, E.P; Gale, N; Baglioni, P; Lehn, J-M; Albinsson, B; Lincoln, P; Wilhelmsson, L.M; Brown, T & Nordén, B; (2007), Addressable high-information-density DNA nanostructures, *Chemical Physics Letters, 440*, 125-129.
4. Web-Server of the Department of Chemistry & Biochemistry, University of the Sciences in Philadelphia, *http://tonga.usip.edu/gmoyna/biochem341/lecture37.html*, [Visited: 100325].
5. Tumpane, J; Kumar, R; Lundberg, E.P; Sandin, P; Gale, N; Nandhakumar, I.S; Albinsson, B; Lincoln, P; Wilhelmsson, L.M; Brown, T & Nordén, B; (2007), Triplex Addressability as a basis for functional DNA nanoconstructs, *Nanoletters,7,12*,3832-3839.
6. Tumpane, J; Lundberg, E.P; Wilhelmsson, L.M; Brown, T & Nordén, B; (2008); Addressable molecular node assembly- Functional DNA nanostructures, Nucleic Acids Symposium, 52, 97-98
7. Wintree,E; Liu,F; Wenzler,L.A Seeman, N.C; (1998); Design and self-assembly of two-dimensional DNA crystals, Nature 394, Macmillan Publishers Ltd.
8. Iqbal, A; Wang, L; Thompson, K.C; Lilley, D.MJ & Norman D.G; (2008), The structure of cyanine 5 terminally attached to double stranded DNA: Implications for FRET studies, *Biochemistry, 47*, 7857-7862.
9. Lakowicz, J.R.; (2006); *Principles of fluorescence spectroscopy*, 3rd ed, Kluwer Academic/Plenum.
10. Blackburn, G., Michael & Gait, J., Michael, (1990), *Nucleic acids in chemistry and biology*, 298, Oxford University Press.
11. Norman, D.G; Grainger, R.J, Uhrín, D & Lilley, D, M.J; (2000), Location of Cyanine-3 on double stranded DNA: Importance for fluorescence resonance energy transfer studies, *American Chemical Society*, 6317-6324.
12. Binder, Alexander, (1997), Agarose Gel Electrophoresis, *http://www.uni-graz.at/~binder/thesis/node41.html*, [Visited: 100125].
13. Berger, S. A., Goldsmith, W. & Lewis, E. R, (2000), *Introduction to Bioengineering*, 421, Oxford University Press
14. Molecular station; *http://www.molecularstation.com/agarose-gel-electrophoresis/*, [Visited: 100125].
15. Moreira, B.G; You, Y; Behlke, M.A & Owczarzy, R; (2004); *Effects of fluorescent dyes, quenchers and dangling ends on DNA duplex stability*; Elsevier Inc.
16. Armitage, B.A; (2005); Cyanine dye- DNA interactions: Intercalation, groove binding, and aggregation; *Top Curr Chem 253*; 55-76; Springer-Verlag Berlin Heidelberg
17. Seeman, N.C; (2005); Structural DNA Nanotechnology: An Overview; *Nanobiotechnology Protocols; Methods in molecular biology 303*.
18. Lundberg, E.P; El-Sagheer, A; Kocalka, P; Wilhelmsson; M.L; Brown, T & Nordén, B, ,(2010); A new fixation strategy for addressable nano-network building blocks, *Chem. Commun., 46*, 3714 - 3716.
19. Scheffler, M; Dorenbeck, A; Jordan, S; Wüstefeld, M & von Kiedrowski, G; (1999), Self-assembly of trisoligonucleotidyls: The case for nano-acetylene and nano-cyclobutadiene; *Angew. Chem. Int. Ed, 38, No. 22*,Wiley-VCH Verlag.
20. Ned Seemans laboratory homepage; *http://seemanlab4.chem.nyu.edu/* , [Visited: 100125]

Appendix

Sides of DNA-ring

Additionally to the time and temperature studies, the sides of the 6 mer DNA-ring were tested by constructing side 1 to 6 (see table a).

Table a. Sides of DNA-ring.

Side	Oligonucleotides
1.	H3, H4
2.	H4, H1
3.	H1, H5
4.	H5, H2
5.	H2, H6
6.	H6, H3

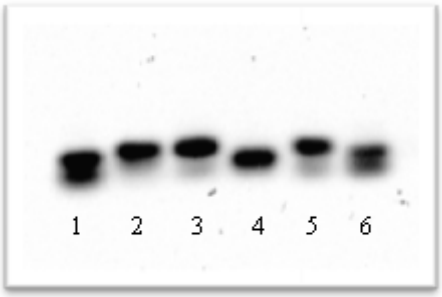


Fig i. Gel image of sides of DNA-ring.
(From left: S1 to S6).

The gel showed that sides 1 and 6 formed poorly, which depends on the H3 oligonucleotide, that is known to be a weak oligonucleotide from earlier studies (fig i).

Comparing heating blocks

When doing the temperature studies, two heating blocks were compared to each other, to see whether this affected the hybridization and thereby the formation of DNA-rings. Therefore DNA-ring with Cy5 modification was studied and samples were put in two different heating QBT1 Grant and QBH2 Grant. It was observed that the latter took longer time to decrease in temperature, thereby affecting the time of hybridization.

Table b. Sample information.

Sample	Temperature (°C)	Time (min)	Heating block
1.	90	5	QBH2
2.	70	28	QBH2
3.	50	40	QBH2
4.	30	180	QBH2
5.	90	5	QBT1
6.	70	33	QBT1
7.	50	40	QBT1
8.	30	180	QBT1

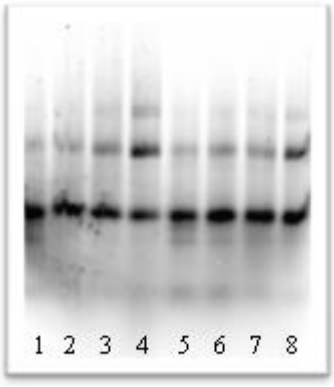


Fig ii. Gel image from comparison between heating blocks (From left: Sample 1 to 8).

As seen in fig ii, the intensity of the monomer bands are very weak, while the intensity of hexagon and 12 mer bands are more pronounced. The measurements of the intensity, as seen in fig iii and fig iv (QBH2 in dark colors and QBT1 in pale colors), show that the heating blocks do differ, but only with a few percent.

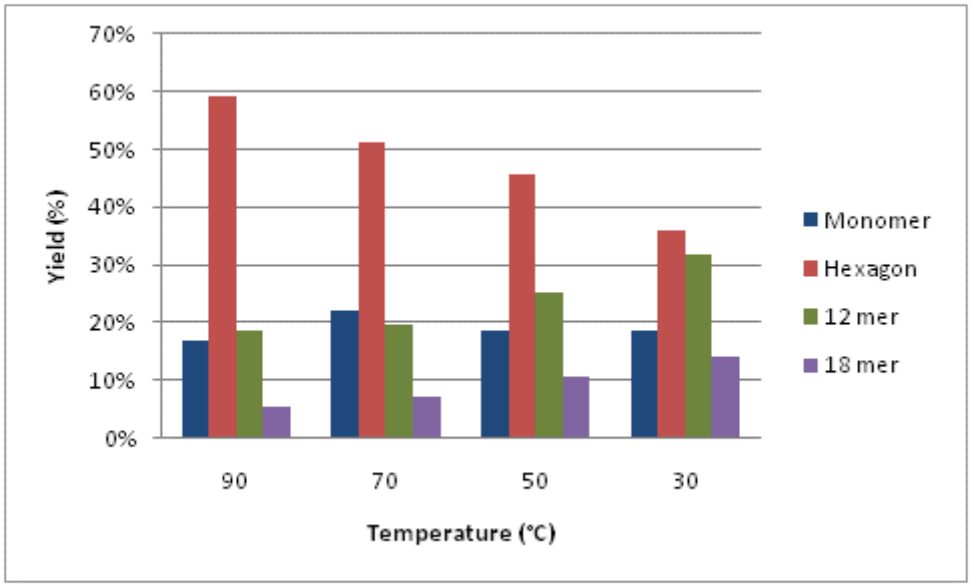


Fig iii. Intensities for heating block QBH2 Grant.

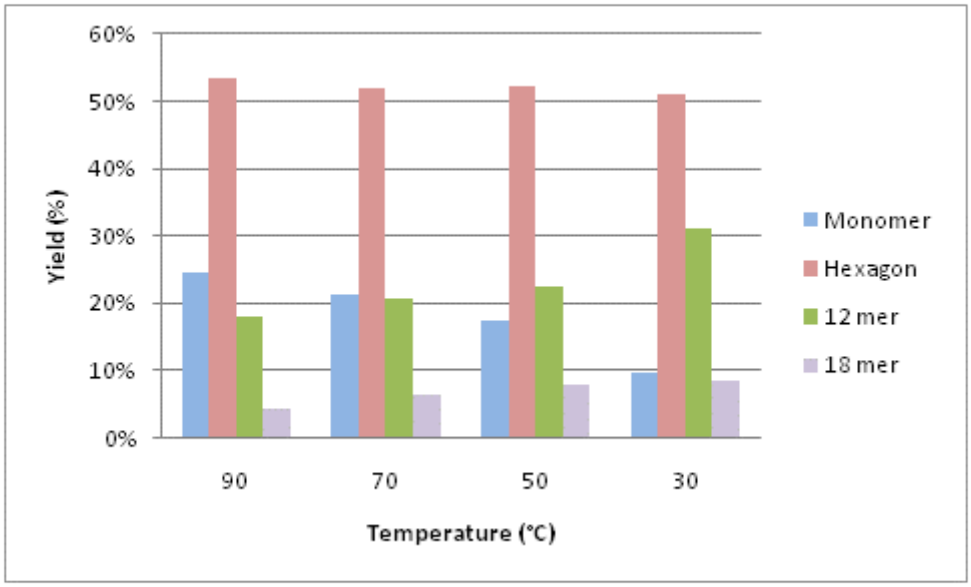


Fig iv. Intensities for heating block QBH2 Grant.

Despite the small difference, it is observed that QBH2 gives a larger formation of the 12 mer and 18 mer DNA-ring, thus indicating that these need more time to form.