Modular assembly of functional DNA-based systems
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Chapter 6
Hierarchical self-assembly of a biomimetic light harvesting antenna based on DNA G-quadruplexes

In this chapter the creation of an efficient artificial light harvesting antenna based on DNA G-quadruplexes is presented. The system possesses covalently attached donor molecules (coumarins) and supramolecularly assembled acceptor molecules (porphyrins). The results demonstrate that the unique structure of the DNA G-quadruplex is crucial for the efficient functioning of the energy transfer system.
6.1. Introduction

Natural light harvesting complexes of the photosynthetic systems present in plants, algae and certain bacteria are the first stage in an intricate and elaborate mechanism to convert light into chemical energy.\[^{[1]}\] These systems contain donor and acceptor molecules that get involved in multiple energy transfer processes. Many elegant covalently assembled artificial light harvesting systems have been developed to help understand the energy transfer processes involved.\[^{[2-4]}\] The synthetic challenge that these systems present is, however, in stark contrast to approaches which utilise self-assembly and self-ordering to form functional artificial light harvesting antenna systems.\[^{[5]}\] Using this latter approach, recent studies have explored the use of biomolecular scaffolds.\[^{[6]}\] The use of biological material as scaffold in the construction of nanoscale light harvesting systems has opened new possibilities in the field due to the ease of assembly. Examples in the literature involve the use of proteins,\[^{[7]}\] viruses\[^{[8]}\] and nucleic acids.\[^{[9-11]}\]

DNA is ideally suited for this approach, since it enables the orderly and predictable modular assembly of DNA strands bearing diverse functionality.\[^{[12, 13]}\] An interesting approach was developed by Armitage and co-workers, which involved the use of branched DNA nanostructures for the development of artificial light harvesting systems (Figure 1).\[^{[14]}\]

![Figure 1. Schematic representation of DNA-based artificial light harvesting antennas using a) non-covalently linked donor and acceptor molecules and b) non-covalently and covalently linked donor and acceptor molecules, respectively.](image)

This approach makes use of different DNA structures as the matrix for donor and acceptor molecules. The combination of non-covalently and covalently anchored donor and acceptor molecules resulted in very efficient energy transfer systems. A key point in the design is the use of DNA as a scaffold that places donor and acceptor molecules in close proximity of each other. The use of non-covalently and covalently bound molecules results in very effective systems. When non-covalently bound acceptor molecules are used, the displacement of donor molecules is required to accommodate them, resulting in a loss in the effective molar absorptivity at the excitation wavelength and therefore, in
lower effectiveness. Furthermore, the variety of possible acceptor molecules is considerably broadened when they are covalently attached since structural requirements for intercalation in the DNA are not further needed. Most recently, the same concept has been applied to three-dimensional DNA structures.\textsuperscript{[15]}

DNA G-quadruplex structures are particularly interesting due to their singular architecture. Their unique structure is the result of the assembly of four guanine rich oligonucleotide sequences stabilized by the presence of monovalent cations (Figure 2).\textsuperscript{[16]}

![Figure 2](image)

**Figure 2.** Top view and schematic representation of a DNA G-quadruplex structure.

The resulting assembled structures provide well-defined binding sites for non-covalent molecular positioning. Furthermore, additional functionalities can be incorporated by covalent modification of the oligonucleotide sequences. DNA G-quadruplex structures, are emerging as versatile scaffolds for functional biomolecular systems,\textsuperscript{[17]} with applications in protein assembly,\textsuperscript{[18]} catalysis\textsuperscript{[19, 20]} and sensing.\textsuperscript{[21, 22]} Its unique structure has inspired the development of new energy transfer systems for analytical purposes\textsuperscript{[23]} and the creation of artificial light harvesting systems with non-covalently bound donor and acceptor molecules.\textsuperscript{[24]} The G-quadruplex approach allows for the rapid assembly of multiple chromophore ensembles with well defined spatial arrangement. Recently, Hamilton \textit{et al.} have reported DNA quadruplex systems that exhibit ultrafast energy transfer between oligonucleotide-tethered pyropheophorbides\textsuperscript{[25]} and G-quadruplexes comprising of statistical mixtures of three different chromophores (Figure 3).\textsuperscript{[26]} The FRET patterns of these mixtures were used for protein recognition. Combined, these reports suggest the potential of using G-quadruplex DNA for the construction of artificial light harvesting systems.
Figure 3. Schematic representation of the guanine-rich oligonucleotides functionalized with three different chromophores for the construction of modified DNA G-quadruplexes developed by Hamilton et al.

6.2. Aim of the research

In this chapter a novel hierarchical self-assembly approach towards the assembly of a functional artificial light harvesting antenna system based on G-quadruplex formation from coumarin (energy donor) modified short oligonucleotides followed by supramolecular binding of a cationic porphyrin (energy acceptor) is investigated (Figure 4).

Figure 4. Schematic representation of the G-quadruplex-based artificial light harvesting antenna. E. T. = energy transfer.

The design takes advantage of the propensity of DNA sequences containing consecutive guanines to assemble spontaneously into stable parallel G-quadruplexes in the presence of K⁺.²⁶ d(GGGTT) oligonucleotides with a covalently tethered 7-methoxycoumarin at the 5' terminus were used. This enabled the formation of a structure that contained a donor chromophore antenna array at one face of the quadruplex. Moreover, the large π-surface presented by the G-quadruplex structure allows for binding of cationic porphyrins²⁷ such as meso-tetrakis(4-(N-methylpyridinium-4-yl))porphyrin (H₂TMPyP4). Importantly,
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however, this approach allows for control of the orientation of the acceptor unit with respect to the DNA bases and the coumarin allowing for efficient energy transfer. Donor and acceptor molecules were chosen based on the significant overlap between the emission spectrum of 7-methoxycoumarin and the Soret band of the absorption of H$_2$TMPyP4 (Figure 5). This overlap is a requisite in the design of efficient energy transfer systems.

![Figure 5. Overlap between the emission spectrum of 7-methoxycoumarin (—) and the Soret band in the absorption spectrum of H$_2$TMPyP4 (---).](image)

6.3. Synthesis of coumarin-DNA conjugates and assembly

The oligonucleotide-coumarin conjugates were synthesized by reaction of 7-methoxycoumarin-3-carboxylic acid N-succinimidyl ester with 5’ amino-modified oligonucleotides d(GGGTT) (Scheme 1). Sequences containing three consecutive guanines were selected to obtain a relatively stable quadruplex structure. Additional thymines were introduced at the 3’ end of the sequence since they are known to prevent quadruplex aggregation.[26]

![Scheme 1. Coupling between the activated ester of 7-methoxycoumarin and amino modified oligonucleotide.](image)

Analogously, coumarin-DNA conjugates using d(GTATT) were synthesized for control experiments. This oligonucleotide sequence should not form G-quadruplex structures when assembled in the presence of K$^+$ since it does not contain consecutive guanines.
The resulting conjugates were purified by size exclusion chromatography (yields were ca. 90%) and characterized by reversed phase HPLC, MALDI-TOF and ESI mass spectrometry.

Coumarin-DNA conjugates forming *(G₃T₂)₄ and non-forming *(GTATT)) G-quadruplexes were assembled by warming of the DNA conjugate solutions in a K⁺ containing buffer to 93 °C for 15 min followed by slow cooling to 5 °C (Figure 6).

Figure 6. Assembly of DNA G-quadruplex by thermal treatment of *(G₃T₂)₄ conjugates in a K⁺ containing buffer.

The resulting assemblies were analyzed by circular dichroism spectroscopy (CD). The spectra confirmed that *(G₃T₂)₄ formed a G-quadruplex while *(GTATT)) did not, as expected (Figure 7). Furthermore, the positive ellipticity maximum observed at 265 nm and the negative minimum at 240 nm for *(G₃T₂)₄ indicates that the G-quadruplex is adopting a parallel structure, thus placing the coumarin array on one side of the quadruplex.

Figure 7. Circular dichroism spectra of G-quadruplex forming (---) ([*(GGGTT)] = 3.2 μM) and non-quadruplex forming (—) ([*(GTATT)] = 3.07 μM) coumarin modified assemblies.
6.4. Binding of acceptor molecules

The interaction of H$_2$TMPyP4 porphyrin with the quadruplex was investigated by a UV/Vis spectroscopic titration. Figure 8 shows the spectral changes upon addition of quadruplex to a solution containing H$_2$TMPyP4.

![Figure 8](image_url)

**Figure 8.** UV/Vis absorption spectra of H$_2$TMPyP4 in an aq. KCl buffer in the presence of increasing amounts (0-2.3 eq) of quadruplex *(G$_3$T$_2$)$_4$. Spectra are corrected for dilution.

The substantial bathochromic shift (14 nm) and hypochromicity of the Soret band (59%) are indicative of a π-stacking interaction between the porphyrin and the G-quadruplex what suggests the porphyrin binding by end stacking or intercalation.$^{[30, 31]}$ Although the binding mode of H$_2$TMPyP4 to G-quadruplexes is a controversial issue, external stacking at the top or bottom ends of the quadruplex is reported to be energetically more favorable than intercalation.$^{[32]}$ The π-stacking interaction is supported by the negative induced CD band at 440 nm observed in the presence of 1 equivalent of porphyrin with respect to the quadruplex (Figure 9).$^{[33]}$

![Figure 9](image_url)

**Figure 9.** CD spectra in the absence (—) and the presence (---) of H$_2$TMPyP4. [*{(G$_3$T$_2$)$_4$} = 32 μM, [H$_2$TMPyP4] = 32 μM.**
Interestingly, in addition to the induced CD observed for the porphyrin there is a strong induced CD for the coumarin at around 360 nm also, which is not present in the absence of H$_2$TMPyP4. This indicates that binding of the porphyrin results in a more defined orientation of the coumarin units that places them within the chiral environment of the G-quadruplex.

The binding of H$_2$TMPyP4 to the DNA G-quadruplex was investigated by the method of continuous variation analysis (Job plot) (Figure 10). Although the data does not allow for unambiguous assignment of the binding stoichiometry, it can be concluded that > 1 eq. of H$_2$TMPyP4 can bind, which is in agreement with observations reported previously.\[30\]

Figure 10. Job plot for the binding of H$_2$TMPyP4 to *(G$_3$T$_2$)$_4$.

UV titrations in which the non-forming G-quadruplex conjugate was used (*GTATT)) showed a considerable hypochromicity of the Soret band (81%) and a bathochromic shift (16 nm) (Figure 11) indicating that, despite the fact that the G-quadruplex is not present, the porphyrin was interacting with the coumarin-d(GTATT) oligonucleotides. Although the kind of interaction between the porphyrin and the modified oligonucleotides cannot not be determined from the data, π-stacking and electrostatic interactions are possible. It is interesting to notice the splitting of the Soret band of the porphyrin that indicates the coexistence of different species. The information obtained from the UV/Vis spectra is unfortunately insufficient for the assignment of the different species present. Additional studies would be required for this purpose. Raman spectroscopy at 420 nm could possibly provide valuable information. However, the fluorescence of the coumarins would interfere in the measurement. Therefore, Raman studies could be possibly done using unfunctionalized oligonucleotides. This experiments, although not directly comparable to the functionalized assemblies would bring new insights in the identification of the different species present.
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6.5. Energy transfer experiments

The ability of the self-assembled systems to engage in energy transfer was investigated by fluorescence excitation spectroscopy (monitored at the porphyrin emission at 671 nm). Upon addition of G-quadruplex \((*G_3T_2)4\) to \(H_2\text{TMPyP4}\), the appearance of prominent bands was observed in the excitation spectrum at 346 nm and 260 nm, (Figure 12). This confirms that energy transfer from the donor (the coumarin and the DNA, respectively) to the acceptor takes place.

Titration of the \(H_2\text{TMPyP4}\) with DNA-quadruplex \((*G_3T_2)4\) monitored by fluorescence excitation spectroscopy shows that the intensity at 346 nm excitation maximises at approximately 1 equivalent (Figure 13). Further addition of quadruplex resulted in a slight decrease. These results show that, although multiple equivalents of \(H_2\text{TMPyP4}\) can bind to the quadruplex, the maximum energy transfer is obtained at a 1:1 ratio. Thus, the system only requires a single bound acceptor to operate at highest
effectiveness. These results are consistent with the fact that at lower quadruplex : porphyrin ratio's the maximum capability of the system to absorb at 346 nm, and therefore to emit at 671 nm, is not reached due to the insufficient number of donor molecules. Once the maximum intensity at 346 nm is reached at 1:1 ratio, no variation is expected when an excess of quadruplex is added. The presence of quadruplexes not bound to porphyrin would result in the absorption at 346 nm and subsequent emission at 405 nm (emission of the coumarin) due to the lack of acceptor molecules. The fluorescent intensities observed at 405 nm by fluorescent emission spectroscopy when monitored at the coumarin absorption at 346 nm would be expected to be increasingly higher for > 1 equivalents of quadruplex. The slight decrease in the fluorescence intensity at 346 nm excitation for > 1 eq. could possibly be attributed to aggregation states of the quadruplexes.

![Graph showing fluorescence intensity at 346 nm with increasing amounts of the quadruplex *(G<sub>T</sub>)<sub>2</sub> (filled squares) and the non-quadruplex forming conjugate coumarin-d(GTATT) (filled triangles).](image)

**Figure 13.** Fluorescence intensity at 346 nm with increasing amounts of the quadruplex *(G<sub>T</sub>)<sub>2</sub> (filled squares) and the non-quadruplex forming conjugate coumarin-d(GTATT) (filled triangles).

The efficiency of the energy transfer from the DNA and the coumarin was calculated from the areas of the bands in the excitation and absorption spectra in Figures 8 and 12 (Figure 14). Although the accuracy in the calculation could not be determined, clear differences were observed for different number of equivalents of quadruplex. For < 0.5 eq. of quadruplex the efficiencies in the energy transfer from the coumarin were found to be around 85%. This is most likely caused by the fact that multiple H<sub>2</sub>TMPyP4 molecules can bind, in principle, to one quadruplex. At 0.5 eq. of quadruplex or lower, all quadruplexes have at least two H<sub>2</sub>TMPyP4 bound and, hence, energy transfer occurs most efficiently. For higher amounts of equivalents of quadruplex, a gradual decrease in the efficiency was obtained. It is interesting to observe that although the maximum excitation at 346 nm is reached at 1 eq., 2 eq. of porphyrin are required for an efficient energy transfer. This is not surprising since at 1 eq. the amount of quadruplex with bound porphyrins present is maximum, resulting in more energy transfer. Nevertheless, this does not imply that the energy transfer between donor
and acceptor molecules at this quadruplex : porphyrin ratio takes place most efficiently. This occurs however once two or more porphyrins are bound.

![Figure 145](image)

**Figure 145.** Efficiency of the energy transfer from the coumarin (filled squares) and from the DNA (filled triangles) to H$_2$TMPyP4 with increasing amounts of quadruplex ($^\bullet$G$_3$T$_2$)$_4$.

These results can be explained by taking the intrinsic lifetime of the excitation state of the coumarins into account which is known to be in the range of the nanoseconds.$^{[34]}$

Since the coumarin array is placed on one of the faces of the G-quadruplex, the distance of the acceptor molecules is crucial for an efficient energy transfer. Although porphyrins interacting via end-stacking with the quadruplex would be placed within the Försters radius (*vide infra*), the energy transfer does not take place equally effective to acceptor molecules placed at the end closer to the coumarin array than to the one further away. A porphyrin bound by end-stacking far away from the coumarin array would accept the energy more effectively from donors with longer excitation life times. In contrast, end-stacking on the side of the coumarin array would place the acceptor close enough for highly effective energy transfer from donors with short excitation life times. The results obtained show that when the ratio quadruplex : porphyrin is 1:1 the efficiency of the energy transfer from the coumarins is not optimum. Two porphyrins are required to obtain the highest efficiency. This could suggest that the first porphyrin would be placed further away from the coumarin array while the second one would be accommodated in a closer position on the top of the quadruplex (Figure 15). This would not be surprising considering the hindering effect that the coumarins could have on the top side of the quadruplex. In this way therefore, the binding of the first porphyrin would be favored on the lower side.
Increasing the amount of quadruplex to > 0.5 eq. resulted in a significant decrease in the efficiency. This will result in a distribution of quadruplexes with multiple equivalents, with two equivalents, with one equivalent and without H\textsubscript{2}TMPyP4 bound. Hence, overall this will result in a lower energy transfer efficiency. Interestingly, maximum energy transfer from the DNA was observed when 4 porphyrins are bound. This result would suggest that more than two acceptor molecules could, in principle, bind to the quadruplex, possibly by intercalation. Furthermore, this would indicate that the intrinsic lifetime of the excited DNA bases is so short (in the range of the picoseconds\textsuperscript{35}) that at least 4 porphyrins are required for the most efficient energy transfer. Despite the differences observed in the efficiencies of energy transfer, a definitive conclusion cannot be reached. The accuracy in the calculation could not be determined thus, a cautious interpretation of the results is necessary.

Experiments with amino-modified G-quadruplexes which did not possess tethered coumarins showed an increase of the band at 260 nm in the excitation spectrum, but no increase at 346 nm was observed (Figure 16). This demonstrates that the excitation band at 260 nm is due to energy transfer from the DNA to H\textsubscript{2}TMPyP4.
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Similar values in efficiency of the energy transfer from the DNA were obtained in the presence and the absence of tethered coumarins for > 0.5 eq. of quadruplex, confirming that the coumarins do not contribute to the energy transfer at 260 nm excitation. However, lower efficiencies were observed for < 0.5 eq. of quadruplex in the absence of tethered coumarins (ca. 60%) compared to samples with bound coumarins indicating that, in this case, the coumarins would contribute to some extent to the energy transfer observed at 260 nm (Figure 17).

Finally, since the lifetime of the photoexcited DNA bases is in the picosecond range,\(^{36}\) it can be concluded that the energy transfer process from DNA to H\(_2\)TMPyP4 is extremely fast. The observation of energy transfer from the DNA bases to the porphyrin further supports the hypothesis that the porphyrins associate to the quadruplex through π-stacking interaction.\(^{30, 36}\)

Confirmation that quadruplex formation was key to the construction of an energy transfer system was obtained by equivalent experiments with the coumarin modified oligonucleotides d(GTATT), which are not capable of forming G-quadruplexes. Addition of coumarin-d(GTATT) to H\(_2\)TMPyP4 was monitored by fluorescence excitation spectroscopy. In the excitation spectra monitored at the acceptor emission at 671 nm, no bands corresponding to the coumarin or DNA absorption spectra were observed (Figures 18 and 13) which demonstrates that despite the interaction of H\(_2\)TMPyP4 with coumarin-d(GTATT), energy transfer from the DNA or coumarin to the porphyrin does not occur.
The results presented here demonstrate that the G-quadruplex structure *(G₃T₂)₄ is a suitable scaffold for the assembly of an artificial light harvesting antenna system. Binding of H₂TMPyP4 to *(G₃T₂)₄, most likely by end-stacking at the top and bottom of the quadruplex and by intercalation, places the acceptor in proximity of the donor chromophores.

The Försters radius, which is the distance at which energy transfer is 50% efficient, was calculated using equation 1.

$$R_0^6 = 0.211 \cdot QY_1 \cdot J(\lambda) \cdot \frac{K^2}{n^4}$$

Where $QY_1$ is the quantum yield of fluorescence of the donor in the absence of the acceptor, $n$ is the refractive index of the solvent and $K^2$ is the orientation between the dipoles of the chromophores. When the orientation is not known a value of 2/3 is used, which means that the relative orientation averaged over all donor-acceptor pairs is assumed to be random. $J(\lambda)$ is the overlap integral which is calculated using the emission spectrum of the donor chromophore, normalized to unit area, and the absorption spectrum of the accepting chromophore:

$$J(\lambda) = \int f_D(\lambda) \cdot \varepsilon_A(\lambda) \cdot \lambda^4 \, d\lambda$$

Where $f_D(\lambda)$ is the overlap of the donor fluorescence spectrum expressed in units of $M^{-1} \cdot cm^{-1} \cdot (nm)^4$, $\varepsilon_A(\lambda)$ is the acceptor absorption spectrum expressed in units of $M^{-1} \cdot cm^{-1}$ and $\lambda$ in nm.

The Försters radius was found to be around 15 nm. This indicates that by taking this approach, the binding of H₂TMPyP4 to both possible sites in the G-quadruplex, holds it within the Försters radius for energy transfer from the 7-methoxycoumarin donor units. However, comparison with the non-quadruplex forming conjugate coumarin-d(GTATT) demonstrates that interaction of the DNA with H₂TMPyP4 by itself is not enough to
achieve efficient energy transfer. Indeed, the G-quadruplex scaffold is essential as it provides the required well defined orientation of donor and acceptor molecules to achieve highly efficient energy transfer.

6.6. Summary and conclusions

In this chapter it has been demonstrated that a novel modular artificial light harvesting antenna system can be formed through self-assembly using the remarkable structural properties of the G-quadruplex motif. The light harvesting system described showed high efficiencies in the energy transfer from donor to acceptor molecules due to the particular structure provided by the DNA. The key advantages of the present G-quadruplex based concept include its modular nature and the ease of assembly, which will allow for rapid structural variation and optimization. It is envisioned that this versatile concept will be a prospectful starting point for the design of new energy transfer arrays that are inherently complex systems.

6.7. Experimental section

General remarks

Amino-modified oligonucleotides were purchased from Biotez Berlin, 7-methoxycoumarin-3-carboxylic acid N-succinimidyl ester was purchased from Sigma Aldrich, meso-tetrakis(3-(N-methylpyridinium-4-yl))porphyrine p-Toluenesulfonate (H₂TMPyP₄) was purchased from TCI Europe.

Reversed phase-HPLC analysis were performed on a Shimadzu LC-10AD VP, Waters Xterra Prep MS C18 column (7.8 x 150 mm, particle size 10 μm) using a gradient of CH₃CN/Triethylammonium acetate (TEAA) buffer 50 mM pH 7; gradient: 05/95 0 to10 min, to 35/65 at 60 min, to 70/30 at 65 min. Flow: 1 mL/min for coumarin modified d(G₃T₂); Waters Xterra MS C18 column (3.0 x 150 mm, particle size 3.5 μm) using a gradient of CH₃CN/Triethylammonium acetate (TEAA) buffer 50 mM pH 7; gradient: 05/95 0 to 10 min, to 35/65 at 60 min, to 70/30 at 65 min. Flow: 0.5 mL/min for coumarin modified d(GTATT). MALDI-TOF measurements were done on a Voyager-DE Pro apparatus. (Matrix: 20 μL of a solution of 2, 4, 6- Trihydroxyacetophenone 0.5 M in ethanol + 10 μL of a solution of ammonium citrate dibasic 0.1 M in Milli Q water + 2 μL sample solution in Milli Q water). Concentration determinations were done using a Nanodrop ND-1000 from Thermo Scientific. UV/Vis measurements were recorded on a JASCO V-660 UV/Vis Spectrometer at 25 ºC. UV/Vis titrations and emission/excitation experiments were carried out using quartz cuvettes with a 1 cm path length. Fluorescence measurements were recorded using a JASCO FP-6200 Spectrofluorimeter. Circular dichroism measurements were recorded using a JASCO J-815 CD Spectrometer.
**Synthesis of coumarin-DNA conjugates.** 364 μL of a stock solution of 5’-propylamino modified oligonucleotide (200 μM in H2O) was mixed with 472 μL of Phosphate buffer (200 mM, pH 7.2). To this solution, 120 μL of a stock solution of 7-methoxycoumarin-3-carboxylic acid N-succinimidyl ester (30 mg/mL in dimethylformamide) was added. The mixture was shaken overnight, centrifuged and the coupled product in the supernatant was purified by size exclusion chromatography (SephadexTM G-25 DNA Grade, Triethylammonium acetate 50 mM, pH 7). The product was analyzed by reversed phase-HPLC and MALDI-TOF.

RP-HPLC – retention time = 36 min  
MALDI-TOF (m/z) = 1872 (calcd. 1873)  
ESI (m/z) = 1873.4 (calcd. 1873.28)

![Diagram](image1)

RP-HPLC – retention time = 39 min  
ESI (m/z) = 1831.60 (calcd. 1832.26)

**General procedure for the assembly of G-quadruplexes.**  
The lyophilized coumarin-DNA conjugate was dissolved in 10 mM Tris.HCl, 80 mM KCl, pH 7.3 buffer to obtain a solution 32 μM of G-quadruplex (128 μM of conjugate) as confirmed by CD.[28] The solution was warmed to 93 °C for 15 min and then cooled slowly to room temperature. The solution was stored at 5 °C for 48 h.

**Titration G-quadruplex – H2TMPyP4**

1 mL of a solution 0.8 μM of H2TMPyP4 in 10 mM Tris-HCl, 80 mM KCl, pH 7.3 buffer was titrated with increasing amounts of quadruplex from a 6.14 μM stock solution in the same buffer.

6.8. References


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