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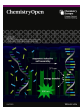

Control of Energy Transfer Between Pyrene- and Perylene-Nucleosides by the Sequence of DNA-Templated Supramolecular Assemblies

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DNA was used as supramolecular scaffold to order chromophores and control their optical properties. Ethynylpyrene as energy donor was attached to 2′-desoxy-2-aminoadenosine that binds selectively to thymidines (T) in the template. Ethynylperylene as acceptor was attached to 2′-desoxyuridine that is complementary to 2′-desoxyadenosine (A). This donor-acceptor pair was assembled along single-stranded DNA templates of different A–T sequences to investigate the sequence control of the energy transfer between the chromophores. The fluorescence intensities increase in the mixed assemblies along the DNA templates from $A_{10}T_{10}$ over $(AATT)_5$ to $(AT)_{10}$, although these templates provide equal numbers of potential binding sites for the two different nucleoside chromophore conjugates and exhibit similar absorbances. This shows that the sequence selective assembly of the two building blocks along DNA templates is programmable and alters the fluorescence readout. Such sequence-controlled supramolecular chemistry represents the key element for future functional π-systems in materials for light harvesting of solar energy.

Multichromophoric systems on the nanoscale are promising nanostructures for light harvesting.[1] This requires precise control of the arrangement of the dyes and distances between them. Supramolecular polymerization offers attractive features to generate such functional chromophore assemblies.[2] DNA is an attractive structural scaffold for supramolecular chemistry because the helical structure, the defined stacking distances between the base pairs and the sequence control encoded by the canonical base allow the specific construction of DNA chromophore architectures.[3] This has been successfully demonstrated for naphthalene-nucleoside[4] and porphyrine-nucleoside conjugates.[5] In particular, the DNA-induced helical twist between the assembled chromophores suppresses the typical self-quenching of the chromophores and control the energy transfer processes.[6] We used DNA as template for the arrangement of chromophore-nucleoside building blocks into multichromophoric systems with interesting optoelectronic properties, which were applied for light harvesting and could be processed in organic electronic devices.[7] With respect to the building blocks it is important that chromophore and nucleoside are linked in a coplanar orientation, e.g. by the ethynylene linker.[8] Hence, the sequence of chromophores[9] and the chirality[10] of such assemblies should be completely programmable by the DNA template.

Recently, we could evidence for the first time the sequence selective chromophore assembly along single-stranded DNA as template, but only by fluorescence quenching. Herein we report on DNA-templated assemblies of pyrene and perylene nucleoside conjugates in which the sequence selective arrangement controls the energy transfer properties between them, and as a result, the fluorescence intensities will not be quenched but altered by the sequence. Pyrene as energy donor was attached by the ethynylene linker to 2′-deoxy-2-aminoadenosine (Py-dAp) which binds to thymidines in the DNA template strand. Perylene as energy acceptor was attached by the same linker to 2′-deoxyuridine (Pe-dU) which binds to 2′-desoxyadenosines in the DNA templates. Both chromophore-nucleoside conjugates form an energy transfer pair.[11]

The syntheses of Py-dAp[9] and Pe-dU[8] were previously reported. The preparation of the supramolecular chromophore DNA assemblies takes advantage of the high solubility of the chromophore-nucleoside conjugates in organic solvents, in particular DMSO, and the low solubility in water. The insoluble conjugates are only kept in aqueous solution by their interaction with the complementary and charged single-stranded DNA template.[7c] Accordingly, Py-dAp and Pe-dU were separately dissolved as stock solutions in DMSO, and were added consecutively to the DNA template in water (Figure 1). Of course, there is an equilibrium between unbound chromophore nucleoside conjugates and those that are bound to the DNA template. The excess and unbound nucleosides are simply removed by two short centrifugations (10 min @ 13,000 rpm). Longer centrifugations remove also formerly DNA-bound molecules. By this procedure, we obtain a maximum of 2% DMSO as solvent in the aqueous solution which is tolerated by the canonical base pairing and base pair stacking.

Different DNA strands were applied as templates to study the sequence selective binding of Py-dAp and Pe-dU and to control the energy transfer properties. To gain insight in the

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An invited contribution to a Special Collection dedicated to Functional Supramolecular Systems

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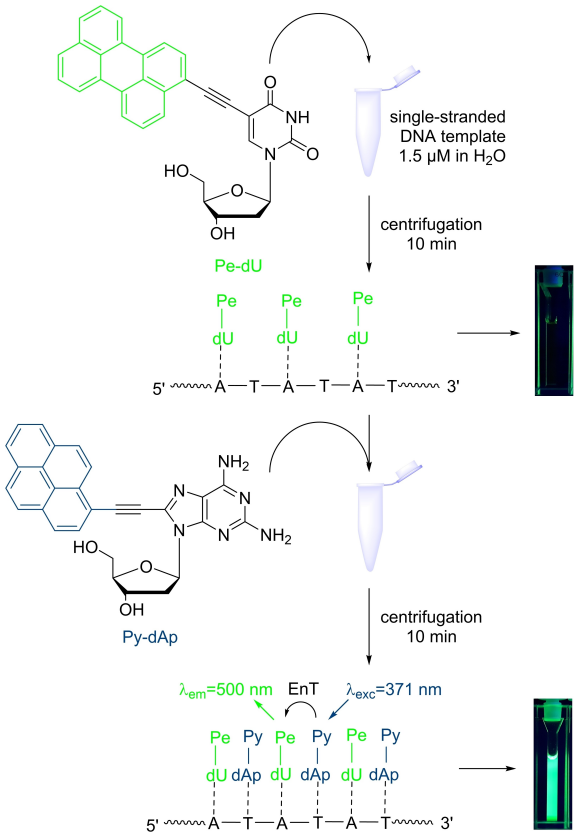

Figure 1. Structure of Pe-dU and Py–dAp, preparation of the sequence selective DNA-templated chromophore assemblies by two consecutive centrifugation steps, and illustration of the energy transfer (EnT) between Py–dAp and Pe–dU in the mixed assembly with alternating sequence along $(AT)_{10}$ as DNA template.

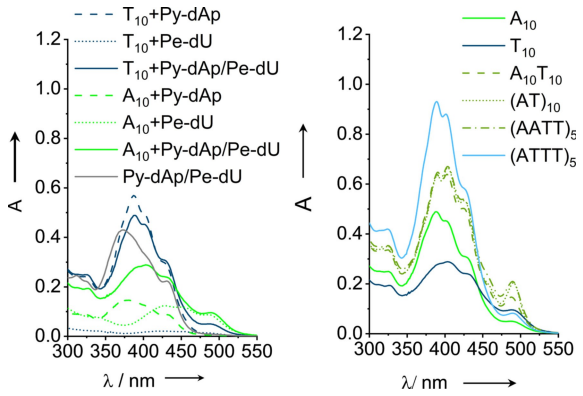

Figure 2. UV/Vis absorbances of the Py–dAp and Pe–dU assemblies in the absence of any DNA template, along T_{10}, A_{10} (left), and UV/Vis absorbances of the mixed Py–dAp/Pe–dU assemblies along $A_{10}T_{10}$, $(AATT)_5$, $(AT)_{10}$ and $(ATTT)_5$ (right) as DNA templates (1.5 μM DNA in water + 2% DMSO).

pure Py-dAp and Pe-dU assemblies, respectively, T_{10} and A_{10} were used as references. $A_{10}T_{10}$, $(AATT)_5$, $(ATTT)_5$ and $(AT)_{10}$ for 20mer mixed assemblies with decreasing sizes of Py-dAp–T and Pe-dU–A clusters and an increasing number of interfaces between the two different chromophore nucleosides. The obtained DNA assemblies were investigated by means of optical spectroscopy, including UV-Vis absorbance, fluorescence, excitation as well as circular dichroism.

In order to get the information which chromophores were bound in the chromophore DNA assemblies, UV/Vis absorbances were recorded. Firstly, we checked the sequence selectivity of Py-dAp and Pe-dU with T_{10} and A_{10} as reference templates (Figure 2 left). The Py-dAp assembly along T_{10} shows a distinct absorption in the pyrene region at 383 nm after centrifugation, whereas the Pe-dU could be almost completely separated according to the low absorbance in the perylene range at 497 nm. That evidences expectedly, that only Py-dAp assembles along T_{10} because it is complementary, whereas the non-complementary Pe-dU does not. The absorbance of the assembly along T_{10} that was prepared by addition of both Py-dAp and Pe-dU is slightly smaller at 383 nm and slightly higher at 497 nm, the characteristic wavelength for Pe-dU, compared to the assembly with Py-dAp exclusively. Obviously, there are some misincorporations of Pe-dU in this T_{10}-guided assembly, which do not follow the canonical rules (e.g. wobble or mismatch pair). Additionally, Py-dAp and Pe-dU are self-complementary, so there is also the possibility of untemplated Py-dAp–Pe-dU pairs in solution if the separation of Pe-dU was incomplete.

Less straightforward is the situation in the experiments with the A_{10} reference template (Figure 2 left). The Pe-dU assembly along A_{10} shows the characteristic perylene absorbance between 420 nm and 520 nm. However, both the assembly with the non-complementary Py-dAp and the assembly with both nucleosides, Py-dAp and Pe-dU, along A_{10} show a significant absorbance in the pyrene range between 350 nm and 420 nm. We assume that this absorbance is not only due to misincorporations of Py-dAp into these assemblies but can mainly be attributed to the slightly better solubility of Py-dAp in aqueous solution due to the second amino group. The solubility was determined based on the sample preparation for the self-assembly experiments and revealed the maximum concentration at room temperature for Py-dAp of 540 μM. Pe-dU has one magnitude of order lower solubility with a maximum concentration of 40 μM. As a result, centrifugation does not efficiently remove the excess and unbound Py-dAp building blocks. This assumption is based on the changed fine structure and the absorbance shift from 383 nm (Py-dAp along T_{10}) to 403 nm (Py-dAp with A_{10}), by the circular dichroism (*vide infra*) and by the fluorescence differences (*vide infra*). In comparison, the absorbance of a sample of Py-dAp and Pe-dU that was prepared in the absence of any DNA template, is even higher than the absorbance of the sample prepared with the A_{10} template. Hence, the presence of the non-complementary DNA strand A_{10} promotes the precipitation of Py-dAp, whereas the presence of the right template T_{10} orders the chromophores into an assembly.

The mixed sequence strands $A_{10}T_{10}$, $(AATT)_5$, $(AT)_{10}$ and $(ATTT)_5$ were used to study the assembly of both nucleosides together and the influence of the sequence on the energy transfer between them. The absorbance at 383 nm mainly results from Py-dAp, and the presence of Pe-dU could be detected by the band at 497 nm. However, the elucidation of

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the sequence selective binding of Py-dAp and Pe-dU along the mixed sequences by their absorbances is difficult since the absorbances of the two chromophores overlap to a certain extent. Nevertheless, the similarity of the absorbances of the mixed assemblies along $A_{10}T_{10}$, $(AATT)_5$ and $(AT)_{10}$ is striking and indicates sequence selective binding only by their different fluorescence intensities (*vide infra*) although each of these three templates provides 10 potential binding sites for Py-dAp and 10 for Pe-dU, respectively. The stronger absorbance of Pe- at 497 nm in the mixed assemblies along $(AATT)_5$ and $(AT)_{10}$ is due to stronger helicity in these arranged Pe-dU conjugates when they are bound as single chromophores (along $(AT)_{10}$) or as chromophore pairs (along $(AATT)_5$). This interpretation is supported by their circular dichroism (*vide infra*) and additionally underscores the sequence selective binding of this chromophore to these DNA templates. The sequence selective binding is further supported by the mixed assembly along $(ATTT)_5$ showing enhanced absorbance at 383 nm and reduced absorbance at 497 nm since this template provides 15 potential binding sites for Py-dAp and only 5 potential binding sites for Pe-dU.

In order to get further insights into the helicity of the DNA-templated Py-dAp/Pe-dU assemblies circular dichroism (CD) spectra were recorded (Figure 3). The CD spectra are dominated by the Py-dAp signals with a positive couplet and an axis transition around 385 nm. Compared to our previously published CD spectra of a DNA double strand covalently labeled with five adjacent ethynyl pyrene groups[12] it can be assumed that the non-covalently assembled Py-dAp units follow a right-handed helicity that is introduced by the single-stranded DNA template. This helicity is observed in nearly all chromophore assemblies of this study, except T_{10} and A_{10} with Pe-dU, because there is no Py-dAp in these samples. In the assemblies along A_{10} with Py-dAp alone and the Py-dAp/Pe-dU mixture there is only a weak CD signal although the Py-dAp absorbances are rather strong for these two assemblies. In particular, a weak bisignate signal with an axis crossing at 403 nm within the pyrene range can only be observed with the Py-dAp/Pe-dU mixture in the presence of the A_{10} template. However, the CD of the Py-dAp/Pe-dU mixture in the absence of any template an altered and not a bisignate signal that cannot be assigned to a helically ordered structure. Thereby CD cannot exclude regular stacking interactions between Py-dAp and A_{10} but the absorbances as discussed above can exclude this. These observations support our interpretation mentioned in the absorbance section above that Py-dAp units remain in solution after centrifugation without assembly along the DNA template. As a result, the absorbance at 383 nm is significant, but the CD is small. The contribution of the Pe-dU units to the CD is rather small but can be seen by the enhanced CD above 420 nm in the mixed assemblies along $(AT)_{10}$ and $(AATT)_5$. Obviously, the helical twist between the Pe-dU units differs from that between the Py-dAp units and causes a smaller CD.

Finally, the fluorescence of the mixed DNA-templated assemblies was recorded ($\lambda_{exc} = 371$ nm, Figure 4). According to the UV/Vis absorbance this wavelength is selective for Py-dAp. The spectra exhibit exclusively the fluorescence of Pe-dU with the perylene typical fine structure and maxima at 502 nm and 536 nm, which is the result of an energy transfer from Py-dAp to Pe-dU. The assembly along T_{10} should consist purely of Py-dAp due to its sequence. The small fluorescence intensity, however, shows that there are few misincorporations of Pe-dU which do not follow the canonical rules and were already evidenced by the small absorbance at 497 nm. In contrast, the assembly along A_{10} shows no significant fluorescence intensity although the rather strong absorbance at 383 nm shows the presence of the Py-dAp units. This is also the case in a sample prepared without any DNA template. As expected based on the absorbance at 383 nm the fluorescence in this sample without any DNA template is even higher because more Py-dAp remains in solution. The absence of fluorescence can only be explained by the spatial distance between the Py-dAp units, presumably unbound in solution as discussed above and the Pe-dU units bound to the DNA template A_{10} which does not promote an efficient energy transfer. In the DNA templates with mixed sequences, the fluorescence intensities increase with the number n of possible Py-dAp/Pe-dU interfaces from n = 1 with $A_{10}T_{10}$ over n = 9 with $(AATT)_5$ to n = 19 with $(AT)_{10}$. It is important to mention here that all three DNA templates provide

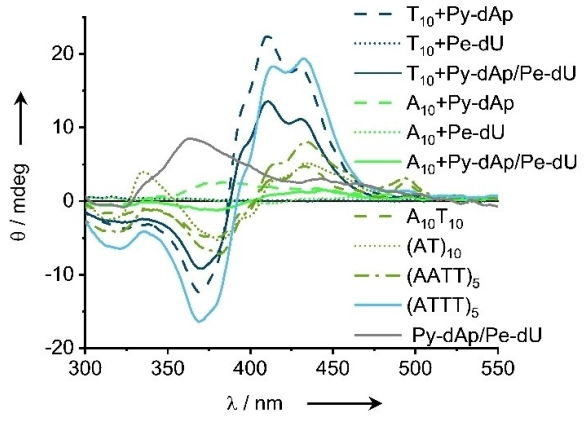

Figure 3. Circular dichroism of the Py–dAp/Pe–dU assemblies in the absence of any DNA template, along T_{10}, A_{10}, $A_{10}T_{10}$, $(AT)_{10}$, $(AATT)_5$ and $(ATTT)_5$ as DNA templates (1.5 μM DNA in water + 2% DMSO).

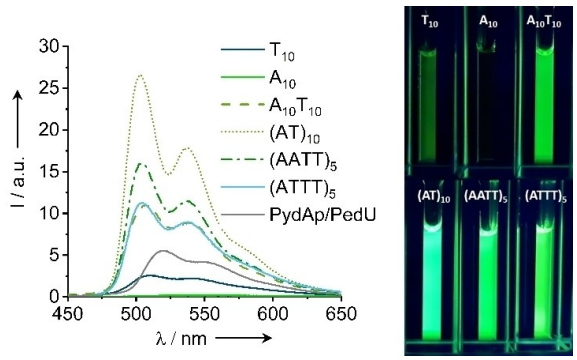

Figure 4. Fluorescence (left) and image (right) of the Py-dAp/Pe-dU assemblies in the absence of any DNA template, along T_{10}, A_{10}, $A_{10}T_{10}$, $(AT)_{10}$, $(AATT)_5$ and $(ATTT)_5$ as DNA templates (1.5 μM DNA in water + 2% DMSO, $\lambda_{exc} = 371$ nm).

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an equal number of potential binding sites for Py-dAp and Pe-dU and accordingly show similar absorbances. The observed differences in fluorescence intensities must be assigned to the sequence differences in these chromophore assemblies. The fluorescence intensity of the assembly along $(ATTT)_5$ is equal to that along $A_{10}T_{10}$, although n is higher (n = 9), because the amount of Py-dAp chromophores as energy donors is higher in this assembly. This underscores our interpretation of a sequence control for the energy transfer between the two different chromophores. Excitation spectra (see supporting Information) support this. Overall, the fluorescence intensity differences in these mixed assemblies are astonishing and show that the optical readout of such mixed chromophore assemblies can be controlled and tuned by the given sequences of the DNA templates. In addition, we showed that the order of addition of the chromophores (1. -dU, 2. Py-dAp, or 1. Py-dAp, 2. Pe-dU) does not significantly alter optical properties of the assemblies (see supporting information).

In conclusion, we showed a programmable non-covalent and sequence selective assembly of two different chromophores, pyrene and perylene, along single-stranded DNA templates (Figure 5). Despite minor misincorporations, the sequence selectivity was evidenced by means of optical spectroscopy and follows the canonical base pairing. The pyrenes were attached to 2′-desoxy-2-aminoadenosine and preferentially bind to thymidines in the template, whereas the perylene modified 2′-deoxyuridines bind to adenosines. Most importantly, the sequence of the DNA template and thereby the sequence of the assembly controls the optical readout. The fluorescence intensities by the energy transfer between the chromophores increase in the mixed assemblies along the DNA templates from $A_{10}T_{10}$ over $(AATT)_5$ to $(AT)_{10}$, although these three templates provide equal numbers of potential binding sites for the two different nucleoside chromophore conjugates and exhibit similar absorbances. This stands in contrast to our previous studies with Py-dAp and a nile red-dU conjugate where the sequence controls fluorescence quenching by energy transfer between the two chromophores.[9] We previously showed that nearly every binding site of single-stranded DNA templates are occupied by chromophore-nucleoside conjugates.[7a,c] These are important results that follow the chemistry principle "structure determines properties". We believe that the sequence programmable arrangement of chromophores in supramolecular chemistry represents the key element for future functional π-systems in materials. In particular, light harvesting of solar energy for photophysics and photochemistry could potentially be improved by the sequence control. DNA is an important structural scaffold to induce this important sequence control.

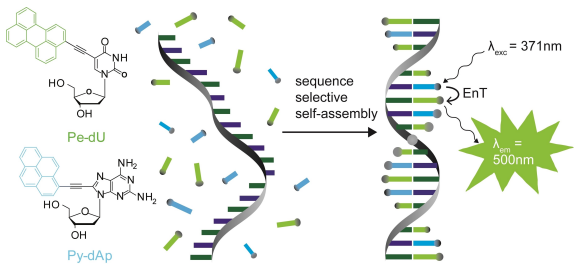

Figure 5. Illustration of the DNA-templated and sequence selective assembly of Pe-dU and Py-dAp to control the energy transfer between them.

Experimental Section

All experimental details are described in the Supporting Information.

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Keywords: oligonucleotides · helicity · circular dichroism · fluorescence · absorbance

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