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Control of helical chirality in supramolecular chromophore–DNA architectures†

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Four different D- and L-configured chromophore–2′-deoxyuridine conjugates were applied to elucidate the helical chirality of their non-covalent assemblies along the D- and L-configured DNA templates by optical spectroscopy. There is no configuration-selective recognition between these nucleosides and the DNA templates. The helicity of the DNA assemblies is either controlled by the configuration of the DNA template or by the nucleoside configuration.

The regular right-handed helical structure of DNA is spontaneously formed by two complementary oligonucleotides and shows well-defined π–π stacking distances between the base pairs and sequence-selective hydrogen bonding encoded by the canonical pairing rules. These two major interactions drive also the formation of the majority of known DNA nanoarchitectures, as pioneered by Seeman and Rothemund,[1,2] in a programmable way.[3–5] The covalent attachment of chromophores to the nucleotides as DNA building blocks adds promising light-harvesting or opto-electronic properties to DNA by structurally designed photo-physics.[6–9] In order to build new and defined chromophore–DNA architectures supramolecular oligomerization of building blocks is the straightforward bottom-up approach.[10] It is synthetically less challenging because it avoids the "bottleneck" of solid-phase oligonucleotide synthesis and purification for covalent connections between the building blocks.[11] For this supramolecular approach, the Watson–Crick base pairing of single-stranded DNA templates together with chromophore-enhanced π–π stacking drives the self-assembly of chromophores. By these means, Schenning *et al.*[12–14] and Balaz *et al.*[15,16] assembled naphthalenes, oligo-*p*-phenylenevinylenes, or porphyrins non-covalently along single-stranded DNA templates. Häner *et al.* even prepared functional DNA-grafted polymers.[17,18] We evidenced the assembly of ethynl pyrenes[19] and ethynyl nile reds[20] as 2′-deoxyuridine conjugates specifically along oligo-2′-deoxyadenosines as DNA

templates to obtain supramolecular DNA–chromophore assemblies as functional photoactive layers in solar cells.[21] Most recently, we showed that ethynyl pyrene and ethynyl nile red can be assembled in a sequence-programmable fashion controlled by the DNA template.[22]

The construction of π-functional supramolecular chromophore assemblies faces the challenge that chromophores have to be kept in close proximity but complete self-quenching has to be ruled out. Helical stacking is an important solution to this problem, and therefore, the helicity is the most important key feature of double-stranded DNA for chromophore architectures, as shown, for instance, with triphenylamines.[23] The helical twist controls the rate and efficiency of energy and electron transfer processes and reduces the complete self-quenching that is typically observed in non-helical aggregates. This principle guided us, for instance, to develop a white-light emitting DNA[18,24] and to use such DNA architectures in solar cells.[21] The key and basic question is how helical chirality is controlled in such supramolecular DNA–chromophore architectures. In principle, chirality may be governed by the 2′-desoxyribofuranoside configuration of the DNA template, which is either D or L. It is known that both unmodified DNA double helices possess the same conformation and dynamic properties except for chirality. The higher order structures of double-stranded L-DNA are also the exact mirror images (left-handed helices) of that of natural D-DNA.[25,26] To our knowledge, however, L-configured chromophore–nucleoside conjugates were not studied before for supramolecular assemblies. Herein, we follow this path and present a new study to elucidate the origin of chirality in DNA-templated chromophore assemblies. We used a combination of two different D- and L-configured chromophor–nucleoside conjugates, **1D/1L** and **2D/2L**, as building blocks which are non-covalently assembled along D- and L-configured DNA templates to study the chirality of supramolecular DNA architectures by means of optical spectroscopy.

The four chromophore–nucleosides differ by the attached chromophore, either ethynylpyrene (**1D** and **1L**) or ethynyl nile red (**2D** and **2L**), and by the configuration of the 2′-deoxyribofuranosides, either D or L (Fig. 1). The D-configured conjugates **1D** and **2D**

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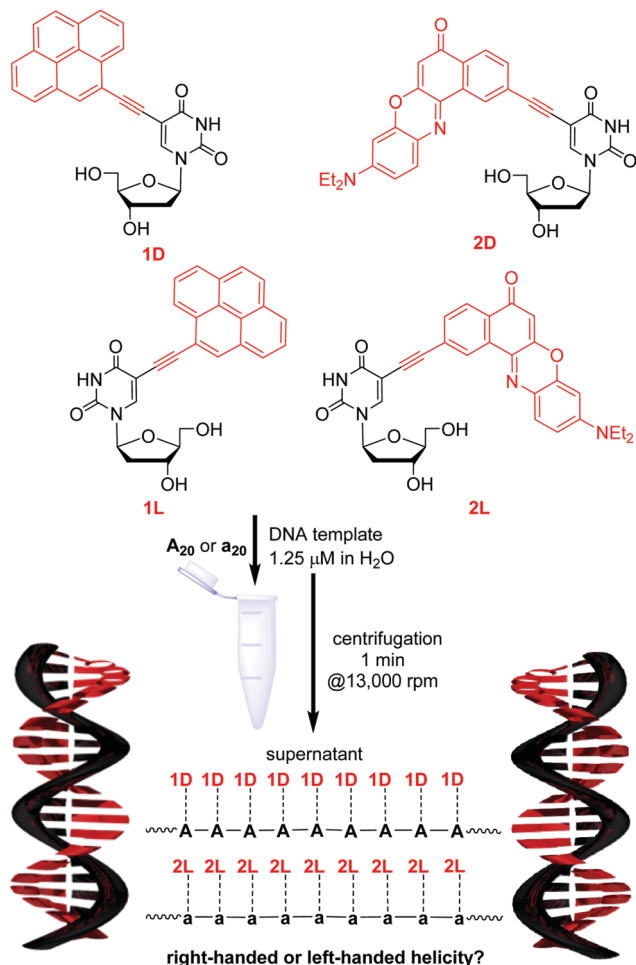
Fig. 1 Structure of **1D**, **2D**, **1L** and **2L**, and preparation of chromophore assemblies along single-stranded DNA templates A_{20} (with D-configuration) and a_{20} (with L-configuration), and idealized schematic drawing of the assemblies of **1D** along A_{20} and of **2L** along a_{20}.

were described previously;[19,20] the L-configured conjugates were synthesized accordingly (see ESI† and Fig. S2 and S3). Stock solutions in DMSO were prepared for each of the four nucleosides. The absorbance and fluorescence properties of the new L-configured nucleosides were nearly identical (within the experimental error) to those of the D-configured ones (see Fig. S16–S19, ESI†). The DNA-templated self-assemblies were prepared according to the following procedure (Fig. 1). Small amounts of the nucleoside stock solutions in DMSO were added to the aqueous solution containing the DNA templates, which are the D-configured A_{20} or the L-configured a_{20}. Not more than 2% DMSO were added to the final DNA samples in water. Notably, all four nucleoside conjugates were nearly completely insoluble in water, and only those nucleosides that were bound by specific base pairing to the given DNA template were kept in aqueous solution. We know from previous studies that **1D** and **2D** specifically bind only to oligo-2′-deoxyadenosines as correct templates and not to oligo-thymidines as wrong templates.[20] This represents an important advantage for the preparation of our supramolecular chromophore assemblies along DNA templates, because excess, hence unbound

chromophores could simply be removed from the sample by short centrifugation (1 min at 16 000g, Fig. 1). A DNA-typical annealing procedure as previously described[19] is not necessary to form DNA assemblies with nearly complete occupation of binding sites.[20] This indicates a thermodynamically driven assembly. After centrifugation, the supernatants of the samples were studied by means of optical spectroscopy.

Firstly, we checked if the nucleosides show selective binding to the differently configured DNA templates. The DNA templates A_{20} and a_{20} are complementary to all four chromophore–2′-deoxyuridine conjugates. Titration experiments were carried out to determine how many binding sites at these DNA templates are occupied. Each chromophore–nucleoside aliquot was equal to the binding of one nucleoside to the template (see Fig. S22 and S23, ESI†). Expectedly, the absorption of the supernatant indicated high occupancy rates for the assemblies with matching configurations between nucleosides and DNA templates (Table 1): 90% of binding sites on A_{20} are occupied by **1D** and 80% of binding sites on a_{20} are occupied by **1L**. **2D** and **2L** bind even quantitatively to their configuration-matching templates A_{20} and a_{20}, respectively, indicating a stronger π–π-stacking interaction between ethynyl nile red chromophores compared to ethynylpyrenes. Surprisingly, high occupancy rates were also observed for those DNA assemblies in which the configurations do not match between nucleoside and template: for instance, **1D** binds to 85% binding sites available on a_{20}, and even 100% binding sites on A_{20} are occupied by **2L**.

These results conclusively show that there is no (or at least no significant) configuration-selective recognition between these four nucleosides and their templates A_{20} and a_{20}. The DNA assemblies with **1D** and **1L** show only slightly reduced occupancy rates if their configuration does not match with that of the template. A closer look on the absorbance reveal also only very minor alterations indicating also only slight differences for stacking interactions. Based on the knowledge that the new L-configured nucleoside conjugates **1L** and **2L** bind to both A_{20} and a_{20} the specific base pairing was additionally checked with T_{20}, G_{20} and C_{20}. In contrast to A_{20} (and a_{20}), the three "wrong" DNA templates T_{20}, G_{20} and C_{20} are not able to keep a significant amount of **1L** or **2L** in aqueous solution (see Fig. S20 and S21, ESI†). This shows that the binding of **1L** and **2L** to the DNA templates follows the specific base pairing rules as previously evidenced for **1D** and **2D** assembled along A_{20}.[20] Similar to double-stranded DNA, these chromophore assemblies show cooperativity with a melting temperature at approximately 70 °C (see ESI,† for **2D** with A_{20}, Fig. S26).

Table 1 Occupancy fraction f **1D**, **1L**, **2D** and **2L** along the DNA templates A_{20} (D-configuration) and a_{20} (L-configuration) and chirality c of the assembled chromophore helices ("+" = right-handed or "−" = left-handed) after centrifugation in water (r.t., pH 7)

Template	**1D** f (%)	**1D** c	**1L** f (%)	**1L** c	**2D** f (%)	**2D** c	**2L** f (%)	**2L** c
A_{20}	90 ± 9	−	65 ± 7	−	100 ± 10	−	100 ± 10	+
a_{20}	85 ± 9	+	80 ± 8	+	85 ± 9	−	100 ± 10	+

1D shows eximer-like fluorescence in DNA-templated assemblies, whereas the fluorescence of **2D** is completely quenched in the assemblies.[20] Hence, we did not further investigate the fluorescence properties of the other chiralities. More importantly, we probed the chromophore assemblies bound to the DNA templates (after centrifugation) by circular dichroism (CD) spectroscopy in order to gain more information on the helical chromophore chirality. All four applied chromophore–nucleoside monomers alone show no significant CD signal, which is typical for small molecules, although they bear the chiral 2′-deoxyribofuranosides. Moreover, unbound aggregates have been removed by centrifugation during the sample preparation. Taken together, CD spectroscopy selectively probes the assemblies of chromophores along the DNA templates without overlaying signals from potentially present excess, but unbound monomers. Unfortunately, the helicity of the DNA core consisting of the 2′-deoxyadenosines of the templates and the 2′-deoxyuridines of the chromophore conjugates **1D**, **1L**, **2D** and **2L** in these supramolecular assemblies cannot be clearly determined, because the CD spectra in the absorption range of the A-dU base pair core around 260–280 nm is overlaid by the ethynyl pyrene or ethynyl nile red chromophores that also absorb light in this range. But the CD spectra of the ethynylpyrene-2′-deoxyuridine assemblies (Fig. 2) show clear exciton-coupled signals in the typical ethynylpyrene absorption range with two Cotton effects at 370 nm and 413 nm and an intervening axis intersection at 385 nm. The assembly of **1D** along A_{20} serves as first reference and shows positive Cotton effect followed by a negative one. This is opposite to the published CD spectrum of a right-handed double-stranded DNA with five covalently attached building blocks of **1D**.[27] Accordingly, the non-covalent assembly of **1D** along A_{20} can be assigned to a left-handed chromophore helicity. Interestingly, such left-handed chirality was also found by others for binding of Zn(II)-cyclen perylene/naphthalenebisimide conjugates to the same template A_{20} indicating complexer mechanisms of assembly.[28] Moreover, the assembly of **1L** along A_{20} shows also

left-handed helicity, although the configuration of the nucleoside and the DNA template do not match. In contrast, both assemblies of **1D** and **1L**, each along a_{20}, show opposite CD effect and thus right-handed helicity. The CD signal of **1L** with A_{20} is weaker probably than the others due to the lower occupation fraction of 65% in this assembly. This makes conclusively clear, that the chirality of the assembled supramolecular chromophore helix is controlled by the configuration of the DNA template: The D-configured template A_{20} yields left-handed helical ethynyl pyrene assemblies whereas the L-configured a_{20} yields right-handed helical assemblies.

For the assemblies with **2D** and **2L** exciton-coupled CD signals were observed in the ethynyl nile red-typical absorption range, consisting of two Cotton effects at 517 nm and 566 nm, and an intervening axis intersection at 540 nm (Fig. 3). The comparison of the CD of the **2D** assembly along A_{20} with the published CD of a DNA with five covalently attached **2D** building blocks,[29] reveals again left-handed chirality. However, in contrast to **1D** and **1L**, the chirality of the DNA assemblies with **2D** and **2L** is not at all controlled by the configuration of the DNA template. Instead, both assemblies of **2D** along A_{20} (matching) and along a_{20} (non-matching configuration) show left-handed helicity, whereas both assemblies of **2L** along A_{20} and a_{20} show right-handed helicity. Obviously, the stronger π–π-stacking interactions between ethynyl nile red chromophores that gain not only higher occupancy fractions at the templates additionally control the chirality of the helical DNA assemblies. It is known that **2D** stacks in left-handed chiral aggregates even in the absence of any template[29] and thereby overrules the chirality control by the templates A_{20} and a_{20} that was observed for **1D** and **1L**. This result tracks well with an important observation by Schenning *et al.*, that, for larger π–π interactions between chromophores in the assembly, the hydrogen bonding interaction to the template diminishes, and the stacking has stronger influence on the structure, here in particular chirality.[30,31]
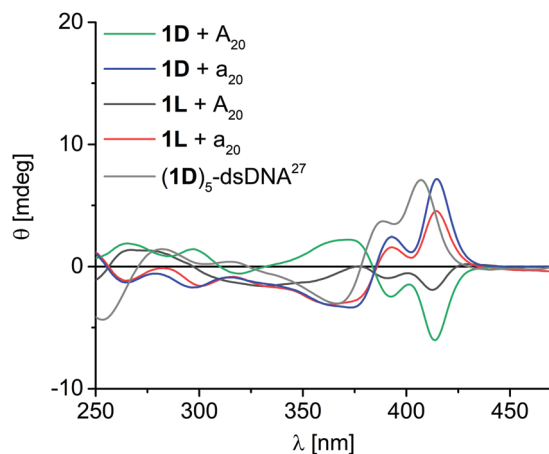

Fig. 2 Circular dichroism spectra of DNA assemblies with **1D** and **1L** along A_{20} and a_{20}; [DNA template] = 1.25 μM, [nucleosides] = 25 μM in water + 2% DMSO, supernatant after centrifugation; grey: double-stranded (ds) DNA covalently modified with five units of **1D** adjacent to each other (2.5 μM in 50 mM Na–P_i buffer, 250 mM NaCl, pH 7.0, r.t.).[27]
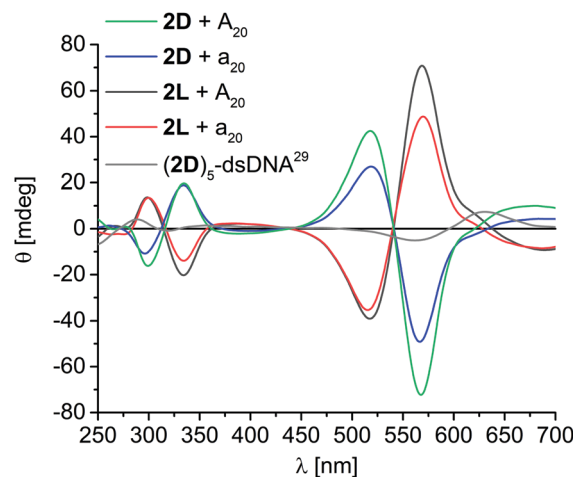

Fig. 3 Circular dichroism spectra of DNA assemblies with **2D** and **2L** along A_{20} and a_{20} templates; [DNA template] = 1.25 μM, [nucleosides] = 25 μM in water + 2% DMSO, supernatant after centrifugation; grey: double-stranded (ds) DNA covalently modified with five units of **2D** adjacent to each other (1.0 μM in 50 mM Na–P_i buffer, 250 mM NaCl, pH 7.0, r.t.).[29]

In conclusion, we studied the way how chirality is controlled in supramolecular DNA-templated chromophore assemblies. We applied a combination of four different D- and L-configured chromophor–nucleoside conjugates, **1D/1L** and **2D/2L**, to probe their non-covalent assembly along the D- and L-configured templates A$_{20}$ and a$_{20}$, respectively. There are several results: (i) the chirality of these supramolecular chromophore–DNA architectures is not simply controlled by the configuration of the DNA template, which could have been expected based on the published D-/L-DNA.[25,26] (ii) Although the selective binding of the chromophore–2′-deoxyuridine conjugates follow specific base pairing rules, there is no configuration-selective recognition by the DNA templates. **1D** and **2D** form not only helical assemblies with their configuration-matching template A$_{20}$ but also with the non-matching a$_{20}$. (iii) The helicity of the DNA assemblies consisting of the ethynylpyrene conjugates **1D** and **1L** are controlled by the configuration of the DNA template. Interestingly, the chirality of these non-covalent assemblies is opposite to the published covalently connected DNA architectures.[27] (iv) In contrast, the ethynyl nile red conjugates **2D** and **2L** form chiral stacks (as previously evidenced also for non-DNA-templated stacks of **2D**)[29] and thus control the chirality of their DNA-templated assemblies. They overrule the control by the configuration of the DNA template, presumably by their stronger π–π-stacking interactions. Taken together, these are important and basic results for the design of DNA-architectures, in particular with respect to their application as emitters and sensors for circularly polarized luminescence.

Conflicts of interest

There are no conflicts to declare.

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