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Aggregation-induced emission by sequence-selective assembly of cyanolated distyrylbenzene in supramolecular DNA architectures†

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Cyanolated distyrylbenzene conjugated to 2′-deoxyuridine is a new building block for supramolecular DNA architectures combining aggregation-induced emission and sequence-selective binding. A high number of binding sites at the DNA template are occupied by cyanolated distyrylbenzenes. Light can be harvested in this assembly and transferred to terminal Atto dyes. Mixed DNA architectures with perylene were programmed by the sequence of the DNA template.

The supramolecular assembly of chromophores into designed hierarchically ordered architectures gives materials with unique optical properties.[1] DNA as a template for such architectures allows the sequence-specific assembly of chromophores *via* the encoded hydrogen bonding pattern.[2] The important features of DNA, chirality and helicity, persist even in the solid state for optoelectronic applications.[3] Organic fluorophores typically show aggregation-caused quenching of their fluorescence in DNA-templated assemblies, *e.g.* of naphthalenes and oligo(*para*-phenylene vinylenes),[4] porphyrines,[5,6] pyrenes,[7–9] perylenes[10] and nile reds.[7,8] An enhanced fluorescence intensity, so-called "aggregation-induced emission" (AIE), is beneficial for optoelectronic applications and was described initially for 1,2,3,4,5-pentaphenylsilole.[11] Since then, there have been more and more examples of differently structured organic chromophores with AIE.[12–14] We recently applied tetraphenylethylene as the most prominent example for non-covalently assembled DNA architectures with AIE.[15] However, only 10–11 out of 20 available binding sites of the DNA templates were occupied by the tetraphenylenes.[16] The propeller-shaped conformation of this chromophore – although planarized in the DNA assembly – sterically hinders and prohibits the sequence-selective DNA assembly in combination with a second chromophore. Herein, we present a cyanolated distyrylbenzene[17,18]-conjugate with 2′-deoxyuridine (**Cs-dU**, Fig. 1(A)) allowing not only a higher occupancy of the DNA template due to its flatter conformation, but also a mixed assembly with a perylene-modified 2′-deoxynucleoside.

Cs-dU was synthesized by a Sonogashira coupling of the iodinated chromophore precursor to 5-ethynyl-2′-deoxyuridine (Scheme S1 and Fig. S1–S6, see ESI†). The ethynylene linker

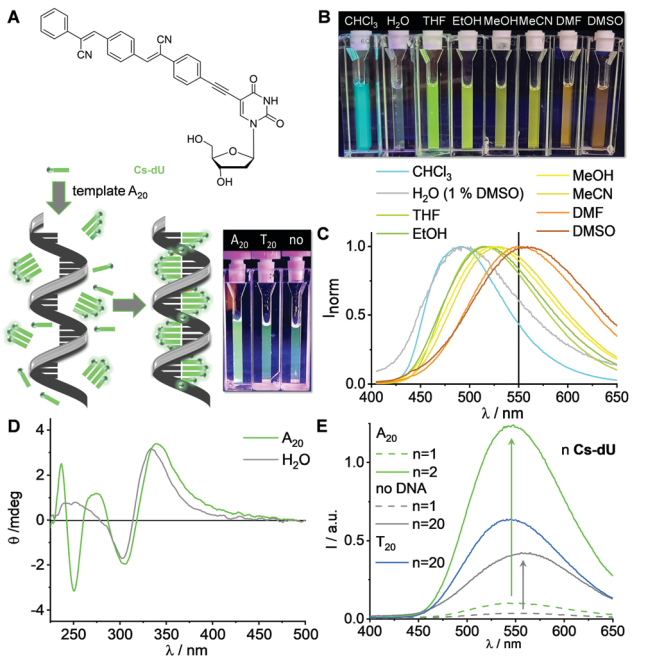

Fig. 1 (A) Structure of **Cs-dU** and illustration of the supramolecular assembly of **Cs-dU** in the presence of A_{20} as a template. (B) Image of the solvatochromic fluorescence of **Cs-dU** using a UV-A handheld lamp. (C) Solvatochromic fluorescence of **Cs-dU** in different solvents; 37.5 μM **Cs-dU**, 1 h at r.t., λ_{exc} = 390 nm. (D) Circular dichroism (CD) of **Cs-dU** without and with A_{20}; 1.25 μM DNA in H_2O (1% DMSO), 25 μM **Cs-dU**, 250 mM NaCl, 1 h at r.t. (E) Fluorescence of **Cs-dU** without and with A_{20} (and T_{20} in comparison), image of the cuvettes using a handheld UV-A lamp (inset in A); 1.25 μM DNA in H_2O (1% DMSO), 1.25 μM (n = 1) and 25 μM (n = 20) **Cs-dU**, 250 mM NaCl, 1 h at r.t, λ_{exc} = 360 nm; for UV/Vis absorption see Fig. S8 (ESI†).

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between the chromophore and 2′-deoxuridine is important because it allows coplanar orientation of the aromatic systems on both sides, which is a prerequisite for the successful DNA-templated assembly.[19] **Cs-dU** shows a strong solvatochromic fluorescence (Fig. 1(B) and (C), Table S1 and Fig. S7, ESI†), typical for this chromophore.[20] Without the DNA template, **Cs-dU** shows a low fluorescence intensity (Φ_F = 0.05, λ_{exc} = 389 nm) probably due to soluble aggregates. The **Cs-dU** obviously assemble in a coplanar order that gives excitonic coupling and helicity according to the observed Cotton effect in the CD spectra (*vide infra*). Based on our experience with other chromophore–nucleoside conjugates (pyrene, perylene, nile red),[7–10] these aggregates must be small, because they cannot be removed by centrifugation. If **Cs-dU** was assembled with A_{20} as a complementary DNA template, there is a strong AIE with a maximum at 550 nm (Φ_F = 0.14). The AIE is significantly smaller in the non-templated assemblies (without any DNA) and with the non-complementary (wrong) DNA template T_{20}, which shows that **Cs-dU** binds preferably and selectively to the dA moieties in the right template A_{20} (Fig. 1(E)), like all our previous chromophore-ethynylene-dU conjugates.[7–9,19] Notably, the AIE with T_{20} shows nearly the same maximum at 544 nm as the AIE with A_{20} at 546 nm and differs from the emission of pure **Cs-dU** in water with a maximum at 558 nm, indicating the effect of hydrogen-bonding to the templates, mismatched with T_{20} and matched with A_{20}. Notably, **Cs-dU** is a modified T and provides hydrogen-bonding to another **Cs-dU**, but the AIE is small compared to the templated assemblies. The combination of the intrinsic stacking of **Cs-dU** in water and the recognition to the template A_{20} that controls further stacking of **Cs-dU** into larger assemblies yields the strongest AIE. In the presence of 15–16 equivalents **Cs-dU** (with respect to the amount of the template A_{20}) the templated fluorescence intensity increase changes to the more shallow linear increase that is also observed in water without any DNA template (see analysis in Fig. S9 and S10, ESI†). This clearly shows that there is additional AIE by the stacking of **Cs-dU** in the assembly along the template A_{20} which cannot be induced in the small aggregates of **Cs-dU** formed without the DNA template. That means additionally that approximately 15–16 out of 20 possible binding sites available on A_{20} get occupied by the **Cs-dU** monomers. This is a significantly higher occupancy degree and thus an improvement compared to the tetraphenylethylene conjugate.[16] These results do not provide any evidence for cooperativity in the DNA architectures. The CD spectra of both the non-templated and the templated samples of **Cs-dU** in water show a positive couplet between 290 and 350 nm with zero crossing at 318 nm (Fig. 1(D)). This reveals that the non-templated, smaller aggregates (as mentioned above) of stacked **Cs-dU** in water exhibit already excitonic coupling and an intrinsic right-handed helicity induced by the 2′-deoxyribofuranoside, which fits then perfectly to the chirality of the DNA template A_{20}. The template extends the size of the **Cs-dU** assemblies, which cannot be seen in the CD spectrum because there is no long-range excitonic coupling. Taken together with the strong fluorescence intensity increase

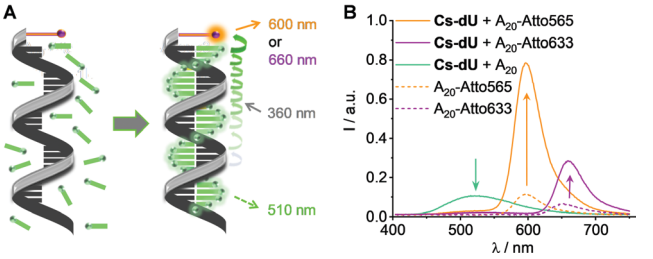

Fig. 2 (A) Formation of supramolecular light-harvesting DNA architectures with **Cs-dU** and the templates A_{20}-Atto565 and A_{20}-Atto633. (B) Fluorescence, 1.25 μM DNA template in water, 25 μM **Cs-dU**, +1% DMSO, 250 mM NaCl, λ_{exc} = 360 nm. For the UV/Vis absorption see Fig. S12 (ESI†).

observed with the template A_{20}, this provides further evidence that stacking of **Cs-dU** in this assembly causes the AIE.

DNA templates A_{20}-Atto565 and A_{20}-Atto633 with the Atto dyes attached to their 5′-termini were applied to prepare DNA-based light harvesting systems with **Cs-dU** (Fig. 2(A) and Fig. S11, ESI†). We assume that the AIE of **Cs-dU** in the assembly along A_{20} is a prerequisite for the energy transfer because it provides significant overlap with the absorbance of the Atto dyes according to Förster. The fluorescence intensity increase that is observed as AIE of **Cs-dU** in the assembly along A_{20} shows that competing non-radiative processes are suppressed which opens the possibility for an energy transfer to appropriate acceptors. Using these supramolecular DNA architectures, the excitation energy is transferred from the planarized and thus emissive **Cs-dU** within the assembly to the terminal Atto dyes as energy acceptors. When **Cs-dU** as an energy donor in the DNA assemblies is excited at 389 nm, the fluorescence of the **Cs-dU** units in both assemblies, with A_{20}-Atto565 and A_{20}-Atto633, at 550 nm is quenched compared to the aggregation-induced emission of the **Cs-dU** assembly with A_{20}. Concomitantly, the fluorescence intensity of the acceptor at 598 nm (with A_{20}-Atto565) and at 660 nm (with A_{20}-Atto633) is strongly enhanced (Fig. 2(B)). The quantum yield of **Cs-dU** (fluorescence range 400–550 nm) is Φ_D = 0.03 with A_{20}-Atto565 (and Φ_D = 0.04 with A_{20}-Atto633) and the quantum yield of the Atto dyes (fluorescence range 550–675 nm) is Φ_{DA} = 0.16 with A_{20}-Atto565 (and Φ_A = 0.14 with A_{20}-Atto633). The energy transfer efficiencies were determined according to $E = 1 - \frac{\Phi_D}{\Phi_A}$, which gives values of E = 81% for the DNA assemblies with A_{20}-Atto565 and E = 72% with A_{20}-Atto633 (Table S2, ESI†). The energy transfer is more efficient than in light-harvesting DNA architectures with 18 pyrenes as the conventional fluorophore for which 56% energy transfer efficiency was observed.[21] Notably, the latter example was based on the pyrene excimer fluorescence in their systems, which complements the use of AIE in our DNA architectures, because excimers are formed only in the excited state and bathochromically shift the pyrene fluorescence. It can be assumed that the energy transfer between the **Cs-dU** units and the Atto dyes in these supramolecular DNA architectures is even more efficient because the fluorescence ranges of **Cs-dU** and the Atto

dyes overlay to a significant extent between 550 nm and 650 nm and thus cannot be completely separated for the calculation of E. Based on the calculated E values, at least 11–13 out of 15–16 DNA-bound **Cs-dU** units are involved and their fluorescence is quenched. Using a regular stacking distance of 3.4 Å, the farest **Cs-dU** involved in energy transfer is located between 51 Å and 68 Å away from the Atto dye at the 5′-terminus. We assume, therefore, that the efficient fluorescence quenching over such distances occurs *via* a step-wise homo-energy transport between the **Cs-dU** units preceeding the final energy transfer to the Atto dyes. This energy transfer mechanism was previously validated for similar supramolecular DNA architectures with tetraphenylethylene-dU covalently incorporated into oligonucleotides complementary to A_{20}.[16]

The fact that 15–16 binding sites of the A_{20} templates can be occupied by **Cs-dU** indicates that the cyanolated distyrylbenzene chromophore is more planar and fits better into the DNA-based assembly compared to the tetraphenylethylene chromophore, where only 11 binding sites were occupied.[16] This property allows us to use **Cs-dU** in combination with **Pe-dAp** as a second chromophore nucleoside conjugate in mixed assemblies (Fig. 3(A)). Such mixed assemblies could not be accomplished with the tetraphenylethylene chromophore. The chromophore sequence in these assemblies is encoded in the sequence of the DNA template, based on the canonical pairing of **Cs-dU** preferably to A_{20} (see above) and **Pe-dAp** selectively to T_{20}.[10,19] The mixed chromophore assemblies were prepared with $A_{10}T_{10}$ and $(AATT)_5$ as DNA templates. Both templates have an identical number of binding sites for both **Cs-dU** and **Pe-dAp**, but different sequences, and identical length to the DNA templates A_{20} and T_{20}. After incubation at r.t. for 1 h, excess of unbound monomers was removed by centrifugation (1 min, 16 000 × *g*, Fig. S13, ESI†). The supernatant contains the DNA assembly with bound chromophores. The mixed DNA assemblies show the characteristic absorption of **Cs-dU** at 323 nm and of **Pe-dAp** at 470 nm and 506 nm (Fig. 3(B)). These absorbances of the mixed assemblies with the DNA templates $A_{10}T_{10}$ and $(AATT)_5$ (each 1.25 μM) have to be compared with the pure assemblies of **Cs-dU** with A_{20} and **Pe-dAp** with T_{20} to obtain enough evidence that mixed assemblies were indeed formed. The absorption of the pure assembly of **Pe-dAp** along T_{20} is A = 0.151 at 506 nm. This wavelength is selective for **Pe-dAp**. If we assume that approximately 15 out of 20 available binding sites at T_{20} are occupied, the absorption A = 0.104 of the mixed assemblies along $A_{10}T_{10}$ and $(AATT)_5$ as templates indicates full occupancy of their 10 available binding sites by **Pe-dAp** monomers. The wavelength 323 nm is not selective for **Cs-dU** because there is significant absorption overlap with **Pe-dAp**. The absorption of the pure assembly of **Cs-dU** along A_{20} at 323 nm is A = 0.369, and the mixed assembly along $A_{10}T_{10}$ has an absorption of A = 0.206. 10 **Pe-dAp** units account for an absorption of maximal ΔA = 0.05 (based on the 323 nm absorption A = 0.081 for the pure **Pe-dAp** assembly along T_{20}). The remaining absorption ΔA = 0.156 at 323 nm clearly evidences that 6–7 out of 10 binding sites at the template $A_{10}T_{10}$ are occupied by **Cs-dU** monomers. Taken together, there is clear evidence for sequence selective assembly of both chromophores into the desired mixed assemblies given by the sequence of the DNA template. The similarity of the UV/Vis absorption of the DNA assemblies with $A_{10}T_{10}$ and $(AATT)_5$ adds even more evidence for the formation of the sequence-selective assembly because both templates provide an equal number of binding sites for **Cs-dU** and **Pe-dAp**, but a different sequence. As a result, we observe an energy transfer between **Cs-dU** and **Pe-dAp** in these mixed DNA architectures. The AIE of the **Cs-dU** units in the pure assembly with A_{20} at 500 nm is completely quenched by the **Pe-dAp** units in the assembly with $A_{10}T_{10}$ and $(AATT)_5$. Instead, an increase of the **Pe-dAp** fluorescence intensity at 538/570 nm as a result of the energy transfer is observed compared to the fluorescence of the pure assembly of **Pe-dAp** with T_{20} although the mixed assemblies with $A_{10}T_{10}$ and $(AATT)_5$ contain a bisected amount of **Pe-dAp** monomers. Based on the sequence-selective assembly dictated by the template, the number of interfaces between **Cs-dU** and **Pe-dAp** is higher in the mixed assembly with $(AATT)_5$ than with $A_{10}T_{10}$. More interfaces improve the energy transfer efficiency; thus, the fluorescence intensity is higher for the assembly with $(AATT)_5$.

In conclusion, we demonstrated that **Cs-dU** is a unique building block for supramolecular DNA architectures. It follows the idea of non-covalent chromophore assembly along the DNA template by stacking interactions between the chromophores and specific recognition by hydrogen bonding to the DNA template, as evidenced earlier by Schenning *et al.*[22,23] Our DNA architectures combine AIE in the non-covalent DNA assembly with energy transfer. The basic idea of our supramolecular DNA architectures with **Cs-dU** is not to simply state that AIE is needed for efficient energy transfer. However, the AIE provides the spectroscopic readout for the successful DNA-templated assembly of **Cs-dU**. The energy transfer pathways are then controlled by the stacked **Cs-dU**s in the assemblies

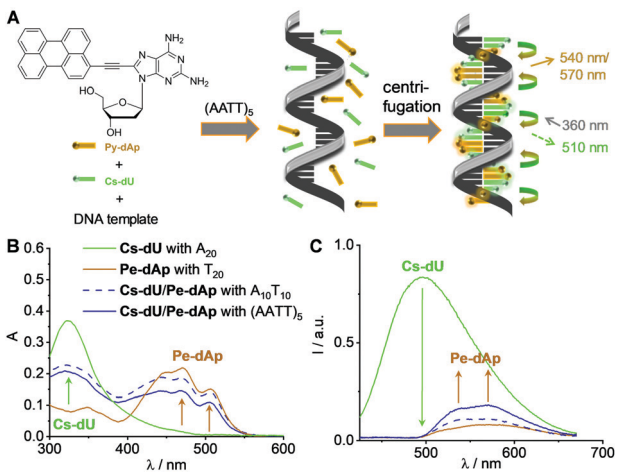

Fig. 3 (A) Structure of **Pe-dAp** and preparation of supramolecular DNA architectures with **Cs-dU**, **Pe-dAp** and $A_{10}T_{10}$ and $(AATT)_5$ as DNA templates. (B) and (C) UV/Vis absorbance and fluorescence of DNA architectures; 1.25 μM DNA template in water, 18.75 μM **Cs-dU**, 18.75 μM **Pe-dAp** +1% DMSO, 250 mM NaCl, λ_{exc} = 360 nm, after incubation for 1 h at r.t. and centrifugation (1 min, 16 000 × *g*).

with the terminal Atto dyes and to the **Pe-dAp**s by their sequence-specific assembly. The rather planar conformation of **Cs-dU** yields a high degree of occupied binding sites at the DNA template achieved with **Cs-dU**, which cannot be realized with the propeller shaped tetraphenylethylenes. This paved the way for the preparation of mixed DNA assemblies with **Pe-dAp.** The sequence of the DNA template does not only program the sequence of the mixed assemblies, but modulates also their energy transfer and optical properties. So far, a precise arrangement of different chromophores in DNA architectures was only possible by covalent incorporation.[24,25] Light harvesting through efficient energy transport within the DNA assemblies of the **Cs-dU** units to 5′-terminal Atto dyes was also realized with high efficiency. The AIE of these supramolecular DNA architectures and their energy transport properties represent new DNA-based materials for technological applications.

HU performed the experiments and wrote parts of the manuscript. HAW supervised the research and wrote the manuscript.

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Conflicts of interest

There are no conflicts to declare.

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