Helical Structures

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Helical Arrangement of Porphyrins along DNA: Towards Photoactive DNA-Based Nanoarchitectures**

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base pairs · chromophores · energy transfer · helical structures · self-assembly

A clear structural scaffold is required for functional π systems if the optical properties depend on the relative orientation of each of the molecular components. The following unique properties of nucleic acids fulfill these requirements:

a) Self-assembly of two oligonucleotides with complementary sequences.
b) Helical duplex structure as a topology with a base-pair distance of 3.4 Å along the axis.
c) Possibility of synthetic modification by automated building-block chemistry.
d) Molecular recognition by nucleic acid binding compounds and proteins.
e) Formation of complex 2D and 3D nanostructures.

Hence it is not surprising that the generation of functionalized nucleic acids is a research topic of increasing interest. The basic idea is to use the self-assembled structure of duplex DNA or RNA as a supramolecular scaffold to arrange multiple chromophores such that they interact with each other in a controllable and predetermined fashion. Such clusters of chromophores are expected to show properties that differ significantly from the monomeric state and can be applied in nanotechnological and biotechnological applications. In principle, multichromophore stacks in oligonucleotides can be structurally realized by 1) base substitutions, 2) sugar modifications or 3) base modifications. In the first part of this Highlight, a few representative multichromophore structures of each type are briefly introduced. Subsequently, the main topic, the helical arrangement of a multiporphyrin stack based on the DNA architecture, is discussed.

Kool and co-workers investigated DNA in which the natural bases were replaced by fluorophores as C-nucleosides, the so-called fluorosides (**A**).[1] Remarkably, the optical properties of stacked fluorosides inside DNA differ significantly from the monomers and show enhanced molar extinction coefficients and enhanced Stokes shifts up to 220 nm, and a combinatorial library of fluorosides yielded a huge range of emission colors.[2] In a different set of experiments, Leumann and co-workers observed strong zipperlike interstrand stacking interaction between C-nucleosides with biphenyl and bipyridine as the base substitutions (**B**)[3] and applied these artificial base pairs for electron-transfer experiments.[4]

Instead of the natural 2′-deoxyribofuranoside, acyclic linkers can be applied between the phosphodiester bridges for the incorporation of chromophores. Asanuma, Komiyama, and co-workers reported the aggregation of azobenzene-derived dyes inside DNA that were incorporated using D- or L-threoninol as the acyclic linker (**C**).[5] DNA hybridization was modulated by photoswitching between the *cis* and *trans* forms of the azo bridge. We have studied the self-organization of perylene bisimides in DNA by incorporating them using (*S*)-aminopropanediol as the acyclic linker (**D**).[6] The hydrophobic stacking of up to six perylene bisimide units inside the duplex yielded a strong red-shifted excimer-type emission. Häner and Langenegger were able to incorporate multiple phenanthrenes and phenanthrolines into DNA using achiral non-nucleosidic linkers.[7] Most remarkably, an achiral oligopyrene stretch in DNA adopts the right-handed helical sense of the DNA framework and forms a corresponding helical chromophore arrangement (**E**).[8]

Alternatively to the strategies above, Nakamura, Yamana, and co-workers have illustrated that the self-assembly of multiple pyrene units attached to the 2′-position of uridine does not significantly influence the stability of the RNA duplex but yields a very strong excimer fluorescence arising from the helical organization of these chromophores (**F**).[9] Moreover, a pyrene zipper array along duplex RNA has been realized by hybridization of two complementary oligonucleotides in which pyrene units were attached to the 2′-position of uridine as well as to the 2′-position of the complementary adenine.[10] Locked nucleic acid (LNA) represents a modified RNA structure in which the ribose moiety is fixed in the RNA-specific 3′-*endo* conformation. Wengel and co-workers studied the applicability of the LNA backbone for the controlled organization of multiple pyrene units by conjugating them to the 2′-function of LNA monomers (**G**).[11]

We have shown that the modification of adjacent 2′-deoxyuridine units with ethynylpyrene yields an increased stability of the DNA duplex arising from excitonic interac-

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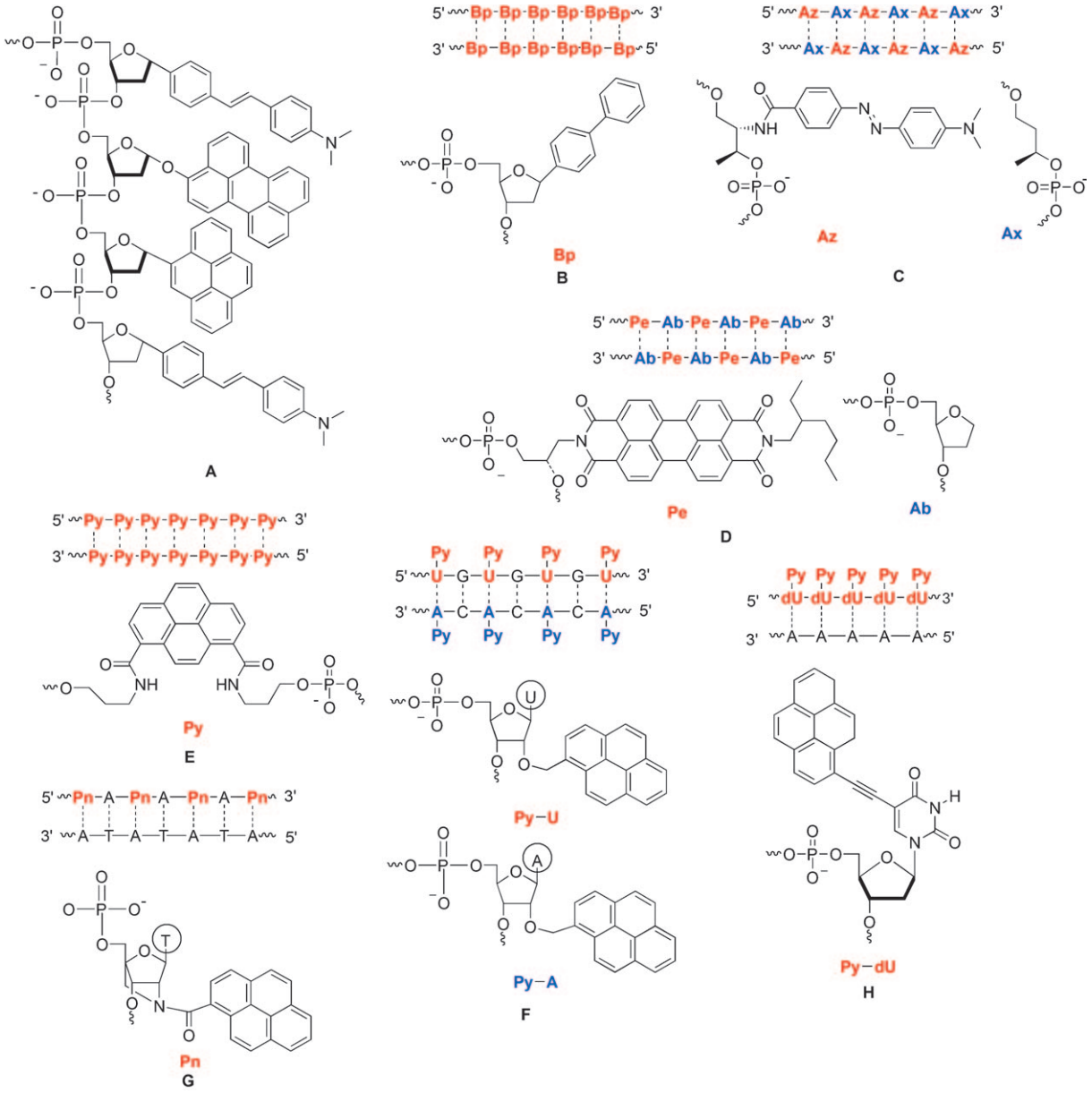

tions only if more than three chromophores are placed in a row (**H**).[12] Furthermore, the interaction of five pyrenes along the DNA helix resulted in a dramatic enhancement (ca. 22-fold) of the fluorescence intensity upon duplex formation.[13] Remarkably, the incorporation of mismatches opposite to the pyrenes destroyed the fluorescence enhancement, as they perturbed the chromophore stack. The emission maximum can be modulated by mixing pyrene and phenothiazine units in the multichromophore.[14]

The latter examples show that it is important to organize the chromophores along the helix such that the canonical base pairing is not perturbed. Preserving the sequence-selective self-assembly of the oligonucleotides despite the presence of the multichromophores is of high significance for nanotechnological applications and for applications in molecular diagnostics, for example, the detection of single base variations. This goal can be achieved by covalent conjugation of the chromophores directly to the DNA bases. It is important to point out that the chromophores need to be attached to the 5-position of pyrimidines or to the 7-position of 7-deazapurines. Otherwise, the modifications would change the *anti* conformation of the modified nucleosides to the *syn* conformation that interferes with the Watson–Crick base pairing. Moreover, attachment of chromophores through short phenylene or acetylene bridges or even single C–C bonds seems to be crucial for the required electronic interactions in the stack.

In the past few years, Stulz and co-workers have studied the self-assembly of porphyrins by attaching them to the 5-position of 2′-deoxyuridine using an acetylene linker.[15–18] The chosen attachment meets all criteria for maintaining the base paring as well as providing strong interactions between the porphyrins, as described above. Moreover, compared to purely organic chromophores discussed earlier, porphyrins have the advantage that metal ions can be inserted into the

chromophore stack. Using this approach, up to 11 tetraphenyl-substituted porphyrin units were successfully incorporated into oligonucleotides.[16] However, the melting temperatures revealed a noticeable drop ($\Delta T = 5$–7 K) of the duplex stability per single porphyrin incorporation. The duplex destabilization was additive for each porphyrin modification. That is, no stabilization could be regained by the interactions between the adjacent porphyrins as was observed with pyrenes and other organic chromophores (see above). This result was somewhat surprising, as circular dichroism (CD) studies and energy-minimized structures from force-field calculations showed that the porphyrins are located in the major groove of the DNA double helix with very little perturbation to the overall duplex structure. The situation became slightly better if diphenyl-substituted porphyrins were attached to 2′-deoxyuridines (dU) and inserted into oligonucleotides.[17] A single modification destabilized the duplex dramatically ($\Delta T = -21$ K). In contrast to the tetraphenyl porphyrins, however, the destabilizing effects of several diphenyl porphyrin modifications were not additive. That is, the destabilization per modification was reduced ($\Delta T = 6$–7 K) in a sixfold-labeled DNA, especially if an alternating porphyrin-dU/A sequence was applied in one strand.

Finally, the key trick to regaining duplex stabilization by the interaction of porphyrins was to use alternating sequences of porphyrin-dU and A in *both* complementary strands of the DNA duplex. The porphyrins are still placed adjacent to each other; however, they are now placed in an alternating fashion between the two complementary oligonucleotides. Using this approach, the destabilization could be significantly reduced and finally turned to a stabilizing effect of $+0.5$ K per modification if 11 tetraphenyl porphyrins are incorporated into one duplex (Figure 1). Even in a mixed alternating sequence of five tetraphenyl and four diphenyl porphyrins, the destabilization was small ($\Delta T = -0.1$ K per modification) and hence negligible. Accordingly, the UV/Vis absorption and CD spectra of these duplexes show strong ground-state interactions between the porphyrins placed along the DNA strand. According to modeling studies, the porphyrins seem to arrange in a zigzag fashion, and they stack in pairs of two porphyrins, one from each strand. This arrangement could explain the stabilization effect and stands in contrast to the arrays in which all porphyrins are attached to only one strand.

It is important to note that the alternating arrangement of the chromophores along the DNA helix has the additional advantage that zinc(II) ions could be inserted into one multiporphyrin oligonucleotide and hybridized with the complementary zinc-free multiporphyrin oligonucleotide conjugate. In such a duplex, the fluorescence of the zinc porphyrin is significantly quenched by the metal-free porphyrins through a resonance energy transfer process within the mixed sequence.

These results are important, as they show that it is potentially possible to arrange metal ions as central atoms of porphyrin units in a defined way along the DNA helix without negatively impacting the base pairing and/or the duplex thermal stability. The significance and potential of DNA as a structural scaffold for the helical organization of porphyrins with metal ions over several nanometers was elucidated. Such DNA-based multichromophore architectures have potential

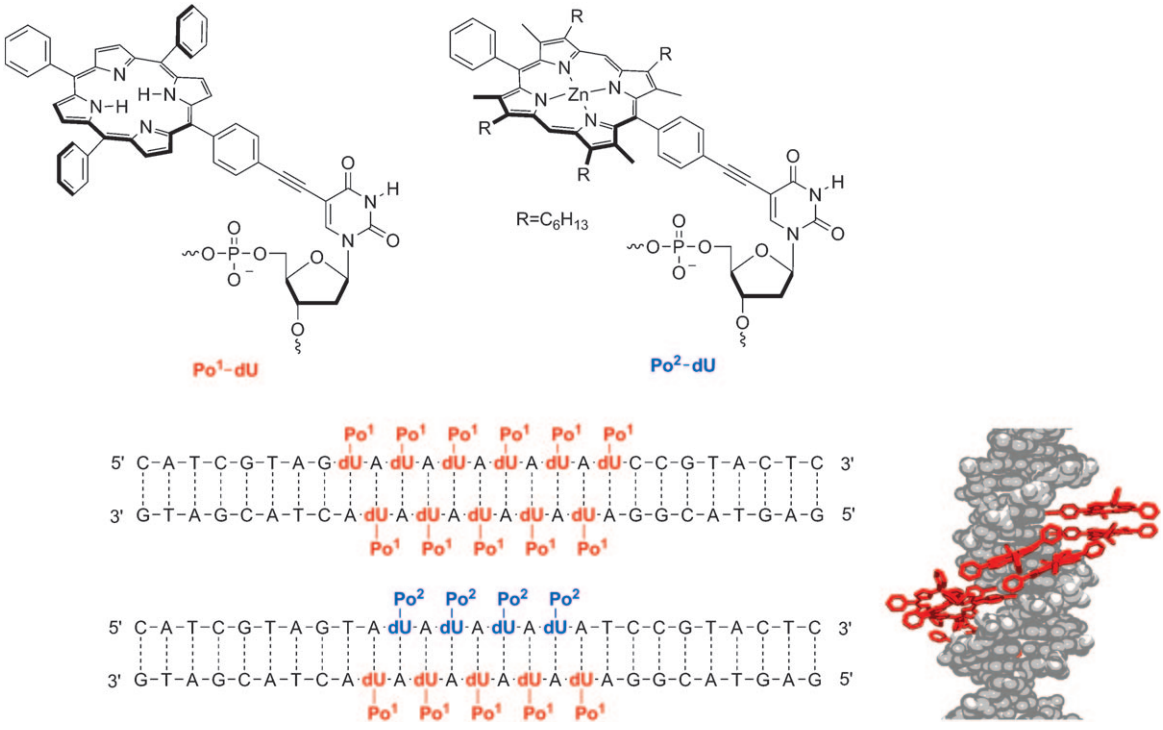

Figure 1. Helical multiporhyrin stacks along duplex DNA: The alternating sequence of porphyrin-dU conjugates and A in both complementary strands places the porphyrin units adjacent to each other in the hybrid.

as functional molecules for photoactive nanomaterials and photonic nanodevices.

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