Nanotemplates

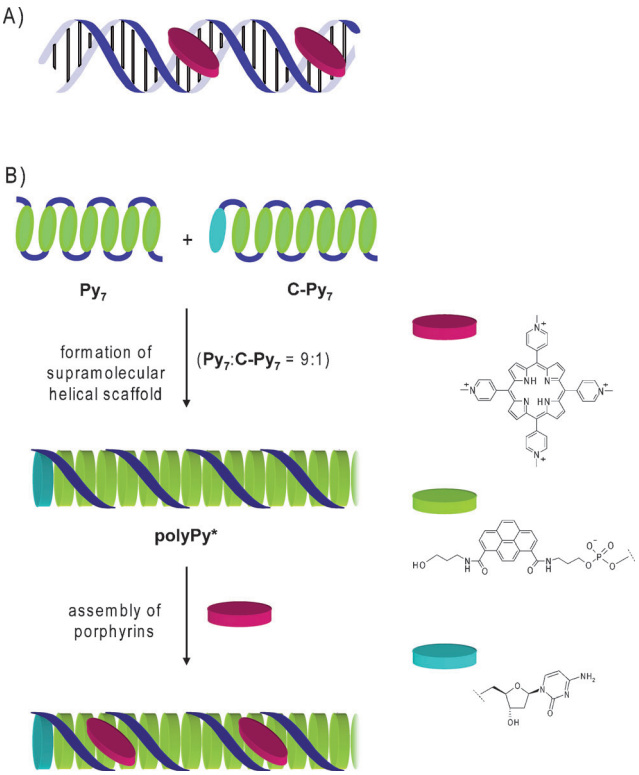
DOI: 10.1002/anie.201201352

Oligopyrenotides: Chiral Nanoscale Templates for Chromophore Assembly**

*Vladimir L. Malinovskii, Alina L. Nussbaumer, and Robert Häner**

The formation of chiral supramolecular assemblies is an important aspect in the development of novel types of materials[1–7] and may find its place in the growing area of nanotechnology.[8,9] In dynamic molecular systems, the occurrence of optical asymmetry may be used to control the electronic signals of modular units.[10–14] DNA can serve as a template for the organization of functional molecules.[15–28] Fundamental studies of DNA–ligand interactions,[29–36] stimulated in earlier days by possible medical applications, were extended to the use of DNA as a chiral template in materials-related studies.[7,15,37,38] Among the many types of compounds investigated, cationic porphyrins take a prominent place.[30,39–44] As a result, the nature of their interaction with nucleic acids is particularly well characterized.[45–48] A wide variety of ligand–DNA complexes has been described. The variability, however, mostly comes from the ligands, whereas changes in the template DNA are limited to the nucleotide sequence. A more fundamental change of the template is a considerable challenge. We recently described the formation of supramolecular helical polymers[49] from anionic pyrene oligomers.[50,51] The close structural similarity of these oligopyrenotides[52] to DNA prompted us to investigate their value as a supramolecular template for noncovalent ligand organization. Herein, we describe the assembly of a cationic porphyrin on an oligopyrenotide scaffold (Scheme 1).

Solutions of supramolecular polymers were obtained after equilibration of a 9:1 mixture of the nonchiral heptapyrenotide Py_7 and its chiral analogue containing an additional, terminally linked nucleotide ($C\text{-}Py_7$) leading to the formation of optically active supramolecular helical polymers by amplification of chirality.[49] Compound ratios, equilibration conditions, and concentrations suitable for spectroscopic analysis were determined in a set of preliminary experiments. A 7 μM concentration of oligomers (corresponding to 50 μM in pyrene units) was found to be ideal and the polymerization process was considered complete within 3 weeks, after which no further changes in the circular dichroism (CD) signals were observed. The cationic porphyrin *meso*-tetrakis(1-methylpyridin-4-yl)porphyrin (H_2TMPyP) was chosen as the ligand because spectroscopic signatures of DNA interactions with



Scheme 1. A) Porphyrin–DNA groove binding interaction[30,43,54] and B) porphyrin assembly on a supramolecular oligopyrenotide scaffold.

this compound have been characterized in depth.[39,40,42,43,46,53–55] Additionally, poly(dA:dT) and poly(dG:dC) served as DNA reference materials for comparison of the obtained spectra.

UV/Vis spectroscopy (Figure 1) provided initial evidence for interaction between H_2TMPyP and the pyrenotide polymer (polyPy*). Addition of the ligand to a solution of the polymers induced a slight redshift (2 nm) of the Soret band. This shift correlates well with the one observed for the binding of H_2TMPyP to poly(dA:dT) in our experiments ($\Delta\lambda = 4$ nm, Table 1) and is in the range of changes described in the literature ($\Delta\lambda \approx 2$–9 nm).[40,56,57] In contrast, a much higher redshift ($\Delta\lambda > 15$ nm) was reported for the complex with poly(dG:dC).[40] Also, the hypochromicity (H, 30%) at the Soret band is in agreement with that obtained for poly(dA:dT) (H, 20%), whereas significantly higher values result from poly(dG:dC) binding (H, 50%).[40] The almost identical band shifts and hypochromisms at the Soret band upon binding of the porphyrin to polyPy* and poly(dA:dT) suggest that 1) H_2TMPyP binds to polyPy* and 2) the interaction resembles the one observed with poly(dA:dT)

[*] V. L. Malinovskii, A. L. Nussbaumer, Prof. R. Häner
Department of Chemistry and Biochemistry, University of Bern
Freiestrasse 3, 3012 Bern (Switzerland)
E-mail: robert.haener@ioc.unibe.ch

[**] Financial support by the Swiss National Foundation (grant 200020-132581) is gratefully acknowledged.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201201352.

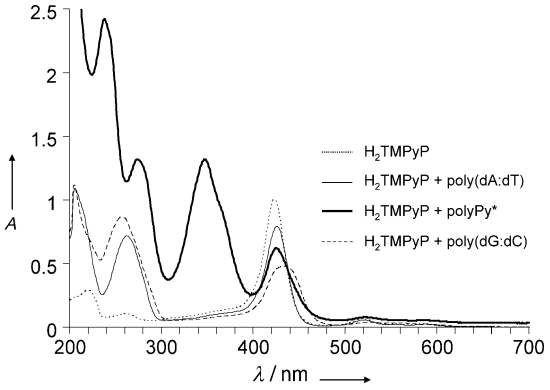


Figure 1. UV/Vis absorption spectra of free H_2TMPyP (4 μM) and H_2TMPyP in the presence of poly(dA:dT), poly(dG:dC), and polyPy*. Conditions: pH 7.0, 10°C; polyPy*: 7 μM total oligomer concentration, DNA: 50 μM in base pairs, 1.0 M NaCl.

Table 1: Spectroscopic characteristics of H_2TMPyP alone and bound to polyPy*, poly(dA:dT), or poly(dG:dC). Conditions: see Figure 1.

	H_2TMPyP	H_2TMPyP + PolyPy*	H_2TMPyP + Poly(dA:dT)	H_2TMPyP + Poly(dG:dC)
Absorbance [λ_{max}, nm]	421	423	425	437
H [%]	–	30	20	50
CD [λ, nm]	–	416	435	445
$\Delta\varepsilon$ [$M^{-1}cm^{-1}$]	–	+21	+15	−7
Fluorescence [λ_{max}, nm]	715	630; 670; 715	655; 715	670; 720

(outside or groove binding) and not poly(dG:dC) (intercalation).

Fluorescence spectra are shown in Figure 2 (and the Supporting Information). Binding of H_2TMPyP to polyPy* leads to an increase of the blueshifted component of the fluorescence signal (Figure 2a). Excitation spectra recorded at two different wavelengths are given in Figure 2b. The two spectra represent the bound and unbound fractions of H_2TMPyP.[58] Energy transfer from pyrene (bands around 250, 280, and 350 nm) to porphyrin is observed for the bound porphyrin ligand but not for the free ligand. The position and the changes of the fluorescence band shape are in agreement with the description of poly(dA:dT) (groove) bound H_2TMPyP.[41,58] Thus, the fluorescence spectra indicate again a similarity of H_2TMPyP complexes formed with polyPy* and poly(dA:dT), but not with poly(dG:dC). In addition, binding of H_2TMPyP to polyPy* does not affect the structure of the supramolecular polymers, since the fluorescence and excitation spectra of pyrenes exhibit no significant changes upon complex formation (see Figure S5 in the Supporting Information). The apparent binding constant for the H_2TMPyP–polyPy* interaction was found to be $(1.4 \pm 0.2) \times 10^5\,M^{-1}$ (pyrene units) as determined by fluorescence titration (see the Supporting Information). Furthermore, analysis of the titration data showed that the number of binding sites at saturation is limited by one porphyrin ligand per three or four pyrene units. The fluorescence data thus show that polyPy* serves as a robust template for assembly of the cationic porphyrins.

Insight into DNA–ligand interactions is provided by CD spectroscopy.[54] Distinct differences in the induced CD (ICD) signal at the Soret band allows differentiation between an intercalative (negative signal) and a groove binding (positive signal) porphyrin–DNA interaction.[43,45] The concentration-dependent appearance of a positive ICD (416 nm, $\Delta\varepsilon = 21\,M^{-1}cm^{-1}$) in this region (Figure 3a) indicates nonintercalative binding of H_2TMPyP to polyPy*.[59] The H_2TMPyP–poly(dA:dT) interaction leads to a similar spectrum (Figure 3b) also exhibiting a positive ICD at the Soret band, redshifted by 15 nm. This shift may originate from structural differences of the two types of polymers. In analogy to the corresponding drug–DNA complexes, we assign the positive ICD to an inclined orientation of the bound ligands relative to the helical axis of the polymer.[59] Therefore, an intercalative binding mode or outside, self-stacked aggregation, which are recognized by a negative ICD or an exciton-coupled CD, respectively,[45,59] can be ruled out. Importantly, the shape and intensity of the exciton-coupled CD in the pyrene region (300–400 nm) remain essentially unchanged, which again demonstrates the structural robustness of the supramolecular scaffold during complex formation. The chiral building block C-Py_7 alone, which was shown previously not to form supramolecular polymers,[49] does not lead to any porphyrin CD signals (Figure S9 in the Supporting Information).

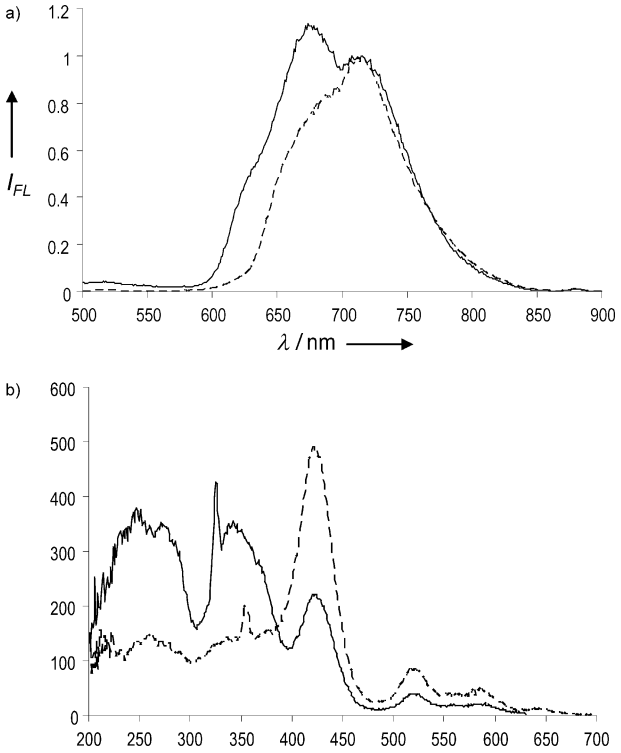


Figure 2. a) Normalized (at 710 nm) fluorescence spectra of H_2TMPyP (6 μM) in the presence (——) and absence (-----) of polyPy*; $\lambda_{ex} = 440$ nm (Soret band). b) Excitation spectra observed at two different wavelengths ($\lambda_{em} = 710$ nm (-----) and $\lambda_{em} = 650$ nm (——)). Conditions: sodium phosphate buffer, pH 7.0, 1 M NaCl, 20°C; 7 μM total oligomer concentration corresponding to 50 μM in pyrene units.

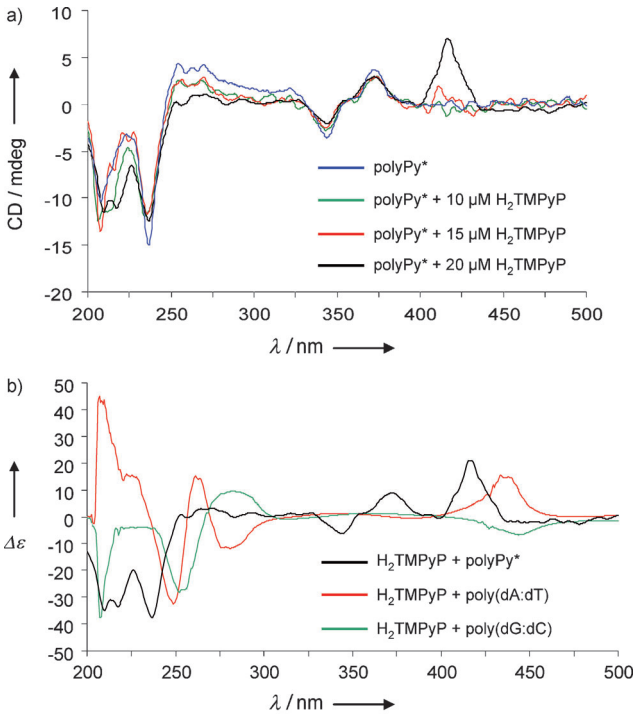


Figure 3. a) Growth of ICD signal in the region of the porphyrin Soret band (400–430 nm) with increasing H_2TMPyP concentration (7 μM polyPy*). b) Comparison of CD spectra of H_2TMPyP (20 μM) in the presence of polyPy*, poly(dA:dT), and poly(dG:dC). Conditions: see Figure 1.

Additional support for binding of H_2TMPyP to polyPy* was obtained from excitation experiments monitoring porphyrin fluorescence at 655 nm (Figure 4), which clearly show energy transfer from the pyrene scaffold to the porphyrin ligands. In addition to direct excitation at the Soret (420 nm) and Q bands (>500 nm), substantial fluorescence also arises from pyrene excitation (250, 280, and 350 nm bands).

The spectroscopic data described above provide strong evidence for the assembly of cationic porphyrins along the helical, supramolecular oligopyrenotide scaffold. Asymmetry is effected by amplification of chirality induced by a small amount of a chiral oligomer.[49] Overall, the asymmetry arises from a single nucleotide, cytidine, which amounts to roughly 1% of the whole polyPy*–H_2TMPyP complex. The binding of H_2TMPyP to polyPy* occurs in a nonintercalative way and closely resembles the groove binding mode of interaction observed for H_2TMPyP–poly(dA:dT). It is reasonable to assume that electrostatic interactions between the polyphosphate backbone of polyPy* and the cationic porphyrin are the main driving force for complex formation. Moreover, the close resemblance of the spectroscopic signatures between H_2TMPyP complexes with polyPy* and with poly(dA:dT) strongly hints at the presence of grooves in the supramolecular polymers, as illustrated in Scheme 1. An additional factor that may, in our opinion, prevent intercalation is the high ionic strength that is required for formation of the supramolecular polymers from the oligopyrene blocks (1M NaCl); it is well documented that high ionic strength (1M NaCl) has a substantial influence on changing the binding mode of H_2TMPyP from DNA intercalation (at low ionic strength) to groove binding.[29,43]

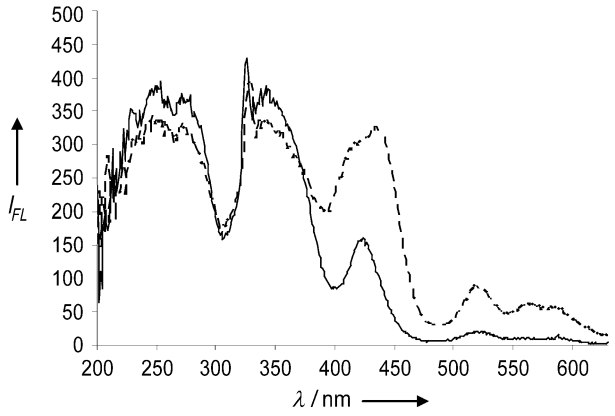


Figure 4. Excitation spectra of 2 μM (——) and 8 μM (-----) H_2TMPyP in the presence of polyPy*. Conditions: λ_{em} = 655 nm; pH 7.0, 20°C, 1.0M NaCl; polyPy*: 7 μM total oligomer concentration.

In conclusion, we have shown that supramolecular polymers formed of short oligopyrenotides serve as helical scaffolds for the molecular assembly of cationic porphyrin ligands. The polymer–ligand aggregate resembles the well-characterized H_2TMPyP–poly(dA:dT) complex. Spectroscopic data favor a model in which groove-bound porphyrins are arranged along a helical, polymeric scaffold in a non-intercalative mode. The study demonstrates the value of supramolecular polymers as templates for the organization of functional molecules. Moreover, they represent alternatives to DNA scaffolds with substantially different electronic properties.

Received: February 17, 2012
Published online: April 11, 2012

Keywords: chirality · DNA · porphyrinoids · supramolecular polymers · template synthesis

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