Post-synthetic DNA modification with porphyrins for DNA templated supramolecular assemblies

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ABSTRACT: The use of azide-alkyne cycloaddition ("click chemistry"), both Cu-catalysed and Cu-free, was probed to attach different porphyrins onto oligodeoxynucleotides (ODNs), and the efficiency was compared to amide coupling reaction. Terminal attachment using the different methodologies provides porphyrin-ODNs in varying yields, and the porphyrin-ODNs can be transformed into multiporphyrin arrays using DNA templated assembly. These arrays show exciton coupling between the porphyrin units and thus demonstrate an efficient and alternative route to multiporphyrin assemblies.

KEYWORDS: porphyrin, DNA bio-nanotechnology, supramolecular arrays, click chemistry, exciton coupling.

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INTRODUCTION

The rational synthesis of porphyrin arrays *via* derivatisation with different substituents to selectively connect porphyrins either covalently or through supramolecular self-assembly has been elaborated well in the past.[1-12] As one of the established templates for supramolecular porphyrin systems, oligodeoxynucleotides (ODNs) proved to be very versatile to arrange porphyrins – and indeed almost any chromophore – in specific three dimensional arrangements.[13-17] This is due to their distinct properties such as: i) predictable three-dimensional structure, in the form of double helix, ii) their programmable nature through rational synthesis, and iii) availability of a range of both commercially and custom-made DNA modifiers.[18] Internal, as well as external modifications can be integrated using a programmed synthesis,[19] which is the most versatile approach. In this respect, internal means that modifiers are within the base-pairing region; external modifiers will be located either in the grooves of the DNA, or at the end of the double helix.

Porphyrins have been attached to DNA by various means, including solid phase synthesis (SPS) and post synthetic modifications;[20] non-covalent approaches have also been explored.[21,22] Due to their aromatic nature, porphyrins are able to be positioned both internally[17,23-26] and externally.[17,24] Examples for internal modification include the seminal works of *Richert et al.* demonstrating successful base–pair replacement with a porphyrin,[27] and *Häner et al.* who have created stacks of porphyrins inside the DNA duplex.[28] Attachment of porphyrins to the periphery of the DNA has been achieved through direct linkage to a nucleobase (most conveniently deoxy-uridine) or the ribose moiety. A range of multiporphyrin arrays have been realised, ranging from dimers to dodecamers,[29-31] where significant exciton coupling[23,32,33] and structure stabilisation[32,34,35] was observed. Such porphyrin arrays have been used in a number of applications, ranging from sensors[36-39] over energy and electron transfer systems[40-43] to photosensitisers[44] and bio-active nano-constructs.[45] One should remember that the hydrophobic nature of the porphyrin tends to lead to intermolecular interactions,[46] but using is as a molecular glue or hook-and-loop fastener (Velcro^{TM}) can actually be useful in the formation of higher order supramolecular structures.

The downside of solid phase synthesis in DNA chemistry is the inherent instability of the phosphoramidite building block. Post synthetic modification of ODNs with porphyrins has been explored, including amide formation,[47] Diels-Alder cycloaddition[48] or thiol–maleimide Michael addition.[49] Even though the use of 1,3-dipolar Huisgen alkyne-azide cycloaddition ("click chemistry") to connect porphyrins to a multitude of functional groups has been explored widely,[50] and despite the fact that is also well examined in DNA chemistry in general,[51-54] only a few reports describe the connection of porphyrins with DNA using click chemistry.[20] These reports include formation of conjugates with metalated tetracationic porphyrins by *Wagenknecht et al*,[48] synthesis of multibranched porphyrin–DNA nano-architectures by *Campidelli et al*,[55,56] attachment of *β*-pyrrolic-modified porphyrins by *Filichev et al*,[57,58] synthesis of a DNA duplex guided porphyrin dimer by *Takada* and *Yamana*,[39] a self-assembled porphyrin–DNA energy transfer array by *Dinolfo* and *Wang*,[59] or attachment of hemin to a G-quadruplex by *Kosman* and *Juskowiak*.[60]

Due to the generally low solubility of porphyrins in aqueous solutions, some of the reported copper catalysed alkyne-azide cycloaddition (CuAAC) reactions were performed on-column while using organic solvents such as DMSO[39,57]. Solution phase click reactions require organic cosolvents in water, such as DMSO. Additionally, CuAAC and strain promoted copper free alkyne-azide cycloaddition (SPAAC),[60] have also been performed in *N*-methylpyrrolidone–water solutions, when DMSO or DMF did not yield any product.[55,59] A water-soluble tetra-cationic porphyrin was also used in CuAAC using DMSO and *t*-BuOH.[48] Yields are only rarely reported[39,58-60] and seem to vary anywhere from <10% to near

quantitative. Reaction times similarly vary greatly with the system, ranging from several days[57,60] over a few hours[48,59] at room temperature to 20 minutes at 70 ºC under microwave irradiation.[58]

In this respect, we have further explored the use of click chemistry to expand the available repertoire of porphyrin-DNA arrays, and find the best conditions by using different derivatives of ZnTPP, and different ways to form azido-porphyrins. We used both CuAAC and SPAAC in solution, and directly compared the coupling efficiency to an on-column amide formation. Short ODNs having a terminal porphyrin modification were subsequently used in the formation of supramolecular porphyrin arrays using a DNA templating approach.

MATERIALS AND METHODS

Full synthetic details of the porphyrins and the ODNs, together with instruments and analytical data, are given in the electronic supporting information (ESI). Alkyne modified DNA was provided either by baseclick GmbH (Neuried; **ODN-1**) or by ATDbio (Southampton, UK; **ODN-2**); **ODN-3** was synthesised in-house (see ESI). HPLC was carried out using a Varian 920-L with a Polaris 3 C18 (150 × 4.6 mm) column at 60 °C, and an aqueous buffer (8.6 mM triethylamine, 100 mM hexaluoro-isopropanol, 0.5 mM EDTA, *p*H 8.3) with a methanol gradient as specified below.

Copper Catalysed Azide-Alkyne Cycloaddition with the Baseclick Oligo-Click Kit

The Oligo-Click Kit was provided by Baseclick, Neuried, Germany. This contained all required reagents, except the porphyrin azides synthesised during this project. Instructions were provided with the kit, but were deviated from slightly. The "activator" (ligand) solution (5 µL) was added to the copper catalyst in the "reactor" vial and thoroughly flushed with argon. To this was added the alkyne-modified oligonucleotide (5 nmoles, 1 equiv.) in water (5 µL) and the porphyrin azide (250 nmoles, 50 equiv.) in DMF (50 µL). The reactor vial was sealed under argon and the reaction mixture was heated to 25 – 30 °C with shaking and in the dark for 24 – 48 hours. After this time no progression in the reaction could be detected by HPLC. When the reaction was deemed to be complete, water (100 µL) was added to the reaction mixture. This was washed sequentially with aliquots of ethyl acetate (200 µL) until the organic layer was colourless. The lack of colour suggested the majority of the free porphyrin had been removed from the vial. The porphyrin-modified oligonucleotide in the aqueous layer was isolated by HPLC. Gradient: 0' (0 % MeOH), 15' (60 %), 30' (70 %), 35' (100 %), 45' (100%). 55' (0%).

Copper-Free Click Chemistry

5'-cyclooctyne-modified DNA (10 nmoles, 1 equiv.) was dissolved in de-ionised water (10 µL) in an argon pursed Eppendorf tube. To this, a porphyrin azide (100 nmoles, 10 equiv.) was added in DMF (40 µL). The reaction mixture was maintained at 25 °C under argon and shaken for 24 hours in a thermomixer. After this time, water (100 µL) was added to the reaction mixture. This was washed sequentially with aliquots of ethyl acetate (200 µL) until all excess porphyrin had been removed. The porphyrin-modified oligonucleotide remained in the aqueous layer and was isolated by HPLC. Gradient: 0' (0 % MeOH), 10' (50 %), 20' (60 %), 25' (100 %), 35' (100%), 45' (0%).

Post Synthetic Labelling of Carboxy Modified DNA with Amino-Modified Porphyrin

The desired oligonucleotide sequences were synthesised using a 1 µM solid phase oligonucleotide synthesis as described in the ESI. The final base added was a Carboxy modifier C10 (Cambio Ltd). This was given an extended 6-minute coupling

time to ensure maximum coupling efficiency. Upon completion of the synthesis, the CPG beads were cut out of the column and collected in an Eppendorf tube. A solution of porphyrin (3 μmol, 3 equiv.) and *N,N*-diisopropylethylamine (DIPEA, 6 μmol, 6 equiv.) in DMF (500 μL) was added to the beads before the tube was flushed with argon and sealed. The tube of carboxy-modified DNA and porphyrin solution was heated to 25 °C, with shaking, in a thermomixer for 18 hours in the dark. The liquid was removed from the tube using a fine pipette to ensure the beads were left behind. These were then sequentially washed with aliquots of DMF (200 μL) until the washes became colourless showing a lack of porphyrin in solution. The porphyrin-modified strands were cleaved from the CPG beads by the addition of ammonium hydroxide (1 mL) and heating to 25 °C in a thermomixer for 2 hours whilst shaking in the dark. The porphyrin coloured solution was transferred to a new Eppendorf tube leaving the now white (or less intensely coloured) beads behind. The solution was heated to 40 °C with shaking and in the dark for 18 hours to deprotect the bases. The crude porphyrin-modified DNA was collected by removing the liquid in an Eppendorf concentrator, and purified by HPLC using the same system as in the copper-free click reaction.

Formation of the ODN assemblies

To assemble the ODN constructs, 2.5 nmoles of the appropriate ODNs were combined in an Eppendorf tube and the sample volume adjusted to 500 μL with PBS buffer (*p*H 7.4). The samples were heated to 90 ºC for one minute, and cooled to 20 ºC at a rate of 0.1 ºC/min. The samples were analysed by UV-vis and steady state fluorescence spectroscopy in 0.1 cm quartz cuvettes. Native gel electrophoresis was performed using acrylamide : bis-acrylamide 37.5 : 1 (20%) and 25 mM Tris–glycine buffer (*p*H 8.8). The gels (10 cm) were run at constant voltage (150 V) for 60 min. The strands were visualised under UV light in BioRad Gel Doc without staining.

RESULTS AND DISCUSSION

Synthesis of the porphyrins

While the availability of azide modified DNA is limited and itself relies on post-synthetic modification, alkyne modifiers are readily available commercially for DNA synthesis. To probe the post-synthetic click modification with our porphyrins, the azido-porphyrin derivatives **P-1**, **P-2**, and **P-3** were synthesised (**Chart 1**, see ESI for synthetic details). Porphyrin **P-1** was obtained by amide coupling of aminopropyl azide to mono-carboxylate tetraphenyl porphyrin (ZnTPP-CO_2H) using 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU) as coupling reagent. The mono-carboxylate porphyrin can easily be obtained by using a six-fold excess of pyrrole and benzaldehyde over 4-carboxy benzaldehyde in a BF_3-catalysed condensation and DDQ induced oxidation procedure.[61] Porphyrins **P-2** and **P-3** were obtained by amide coupling of 5-bromovaleryl chloride to the corresponding amino-porphyrin;[62,63] both adducts were further reacted with NaN_3 to give the azide containing porphyrins **P-2** and **P-3** (see ESI). The transformations proceeded smoothly, showing the versatility of the approach by either using a carboxylate or an amino porphyrin, and additional substituents can easily be incorporated at the *meso*-positions of the porphyrin ring as shown in the structure of **P-3**. The amino-porphyrin **P-4** was obtained by amide coupling of the free-base version of the above–mentioned mono-carboxylate porphyrin with the mono-Fmoc protected diaminopropane and subsequent deprotection with piperidine.

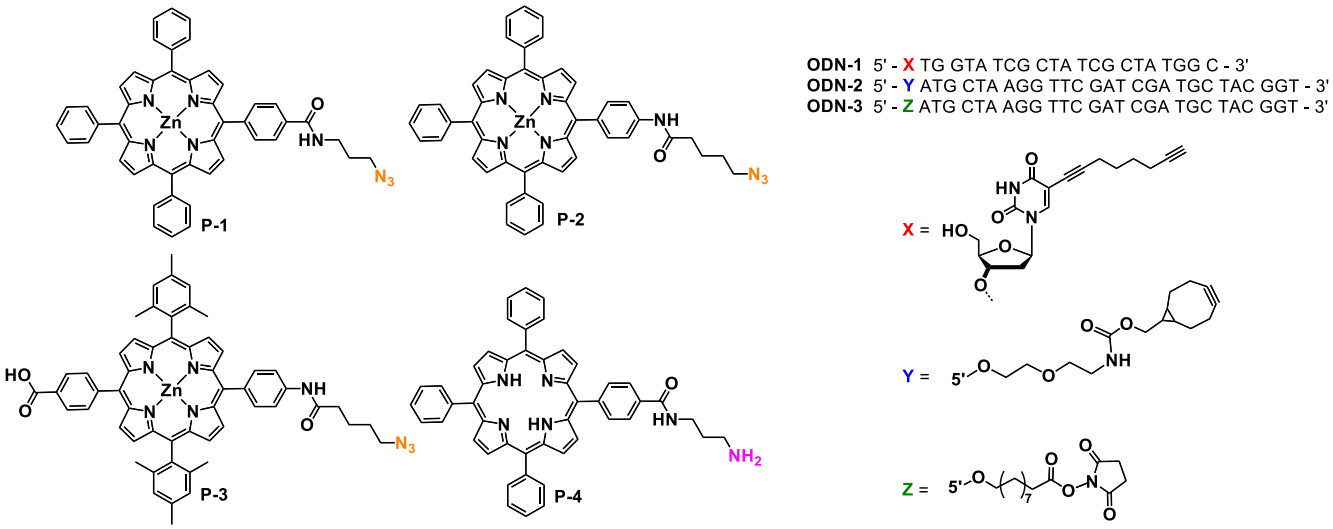

Chart 1. Structures of the porphyrins for post-synthetic modification using click-chemistry (**P-1** to **P-3**) and amide coupling (**P-4**), and ODN sequences including structures of the end-modifiers. The axial ligand on the zinc metal has been omitted for clarity.

Copper-catalysed click reaction on DNA

We first attached porphyrins to **ODN-1** to test the "classical" CuAAC reaction. Solution phase click chemistry between the ODNs and the azido-porphyrins (**P-1**, **P-2**, and **P-3**) was employed. In all cases, zinc metallated porphyrins were utilized in order to avoid the copper metallation of the macrocycle during the CuAAC reactions. The Cu(I) catalysed cycloaddition was performed using the Oligo-Click-Kit from baseclick® containing a solid source of copper and ligand (undisclosed origin and structure).[64] The provided protocol was adjusted to using DMF and water (4:1) instead of DMSO and *t*-BuOH (3:1) as in the latter solvent mixture no product was observed, a fact attributed to the low solubility of the porphyrins in *t*-BuOH. Equally, higher water content in the DMF mixture resulted in precipitation of the porphyrins. Monitoring the reactions (5 nmol ODN and 250 nmol porphyrin in 60 µL of solvent) over the course of five days at 25 °C showed conversion of the alkyne-ODN to the mono-porphyrin adduct; maximum conversion was achieved after 48 hours. Analysis of the crude mixtures using RP-HPLC (**Fig. 1, left panel**), where traces recorded at both 260 nm and 420 nm, showed a characteristic new elution peak at ~16 min (~55% MeOH) with identical R_t for both wavelengths. We found that eluting porphyrin-DNA is best done with 8.6 mM triethylamine / 100 mM hexafluoro isopropanol buffer and a MeOH gradient. The peaks that eluted < 5 min (<10 % MeOH) correspond to either unreacted or degraded ODNs which were not further analysed; unreacted porphyrin eluted at 100% MeOH. The HPLC profiles are consistent in both elution order and peak shapes to what was reported earlier. The products were isolated, and MALDI-ToF analysis confirmed the mono-addition of porphyrin (see ESI). Based on HPLC peak integration (taking the 50-fold excess porphyrin into account) at 420 nm, the conversion was >99% for **ODN-1/P-1**, 95% for **ODN-1/P-2**, and 50% for **ODN-1/P-3**. The isolated yields after HPLC purification were 75% for **ODN-1/P-1**, 39% for **ODN-1/P-2** and 18% for **ODN-1/P-3**. It is worth noting that **ODN-1** showed degradation after more than two days of reaction or at higher temperatures (50 °C). Also, an additional peak could be seen with **P-3** (R_t ~7.5 min); even though this peak seemed to consist of a porphyrin-ODN adduct it could not be identified. Overall, the formation of **ODN-1/P-1** gave the cleanest reaction and highest conversion.

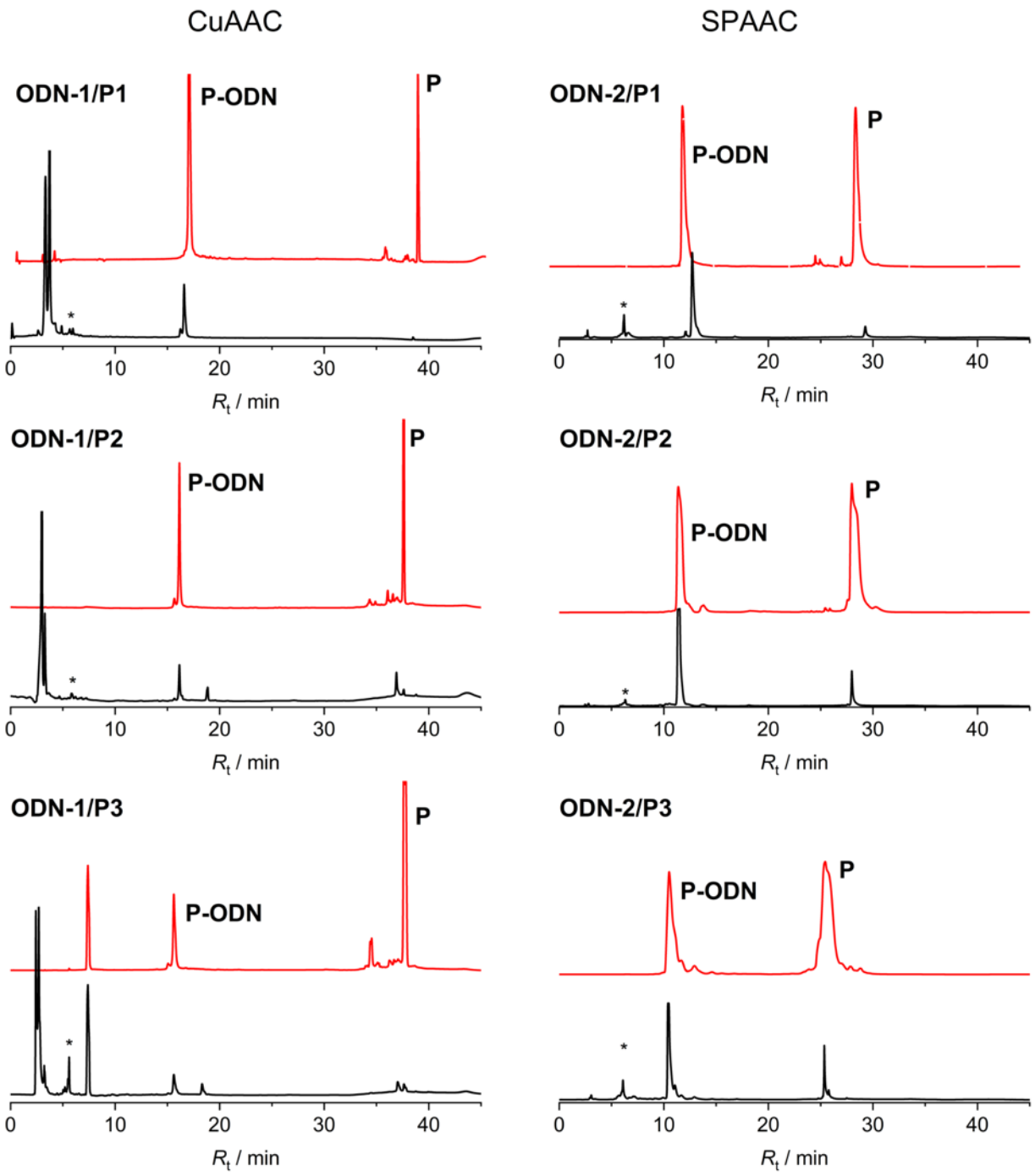

Figure 1. RP-HPLC traces of the crude reaction mixtures using CuAAC (left panel) and SPAAC (right panel) to synthesise mono-porphyrin ODN adducts. Traces recorded at 260 nm (ODN absorbance, bottom black trace) and at 420 nm (porphyrin absorbance, top red trace) are stacked. Peaks eluting at $R_t < 5$ min (< 5% MeOH) in the left panel constitute of reagents and solvent (DMF). Peaks eluting at 10 – 18 min

(50 – 55% MeOH) are porphyrin-ODN (labelled **P-ODN**), and at 25 – 40 min (100% MeOH) are unreacted porphyrin (labelled **P**). Unreacted ODN is labelled with an asterisk.

Copper-free click chemistry on DNA

We then investigated SPAAC through strain promoted alkyne-azide cycloaddition (SPAAC) using bicyclo[6.1.0]nonyne (BCN) as terminal modifier in **ODN-2** (**Chart 1**). We quickly realised that this reaction tolerates a better porphyrin to ODN ratio, namely 10 nmol of **ODN-2** and 100 nmol of porphyrin (in 50 μL H_2O/DMF 1:4). The reaction mixtures were maintained at 25 °C for 24 hours and again analysed by RP-HPLC (**Fig. 1, right panel**). The traces show very little remaining ODN, and mainly porphyrin-ODN with R_t between 10 and 12 min together with unreacted porphyrin (R_t between 25 and 30 min); the differences in R_t compared to the CuAAC reaction arise from the difference in linker moiety between the ODN and the porphyrin, but also from using a different gradient system. Based on HPLC peak integration at 420 nm, the conversion was between 85% and 89% for all three reactions. The isolated yields for the three different porphyrin adducts are 21% for **ODN-2/P-1**, 62% for **ODN-2/P-2** and 48% for **ODN-2/P-3**. The products were again analysed by MALDI-ToF-MS which confirmed the attachment of the porphyrins (see ESI). Noteworthy, the porphyrins show broader peaks due to the faster elution; in our experience this can lead to smearing of the porphyrins on the column.

While the yield for **ODN-2/P-1** was substantially lower as for the CuAAC reaction, the conversions for **P-2** and **P-3** were significantly higher using SPAAC. A possible reason for varying (or even very low) yields in both types of click reactions was suggested to arise from coordination of the azide to the zinc metal centre in the porphyrin.[65] In this work, the binding of azide to zinc was ascribed to the detection of two distinct infrared signals at 2097.1 cm^{-1} and 2122.7 cm^{-1}, where the former was assigned to the free species. The IR spectrum of a reference azido zinc porphyrin (**Figure S3**) did indeed show the same two peaks at 2081.5 cm^{-1} and at 2118.3 cm^{-1}. However, the free base version of this porphyrin also showed two peaks (2087.4 and 2122.8 cm^{-1}). Noteworthy, the higher wave number peak in our azido zinc porphyrin is significantly more intense, whereas in the free base porphyrin they appear with equal intensity. At this point, it is unclear whether zinc coordination is leading to the variable yields, but it cannot be ruled out. Also, we did not attempt to use microwave assisted synthesis[58,66] or different buffers (e.g. TEAA) as we wanted to keep the system as simple as possible for a direct comparison.

Amide coupling to DNA

The successful attachment of porphyrins to ODNs using both CuAAC and SPAAC was then compared to amide coupling to ODNs. We found that the coupling of amino-modified ODNs to a H_2TPP-carboxylate (H_2TPP-CO_2H),[67,68] which was activated as *N*-hydroxysuccinimide (NHS) ester, was not very efficient (DMF / TEA buffer *p*H 8.5). On the other hand, the amino-porphyrin **P-4**, which was obtained by amide coupling with mono-Fmoc protected diamino-propane followed by deprotection with piperidine, could be attached to **ODN-3** containing a 5'-carboxy modifier. In this case, the porphyrin was added through on-column reaction as the carboxy-modifier is not compatible with the cleavage and deprotection protocol for DNA after solid phase synthesis. The synthesis columns were treated with **P-4** in DMF for two hours (using 5 equiv. of **P-4**) where the solution was passed back and forth every 10 minutes with the aid of syringes. Cleavage, deprotection, and purification using standard protocols yielded the **ODN-3/P-4** in 14 %, which is relatively low compared to the click reactions, particularly when compared with **ODN-1/P-1**, **ODN-2/P-2** and **ODN-2/P-3**. The yields obtained from amide coupling with ODNs varies greatly, and we experienced that this reaction can be temperamental, though it clearly is an alternative route to

porphyrin-ODNs. This route is also much more cost-effective when comparing the purchase of amino-modified ODNs to alkyne-modified ODNs and leads to easier scale-up.

Self-assembly of porphyrin-DNA

The possibility for post-synthetic attachment of the porphyrins to ODNs *via* click-chemistry and amide coupling let us to the design of a self-assembled supramolecular porphyrin system as shown in **Fig. 2**. This serves as confirmation that end-modification of ODNs with porphyrins forms a viable route to multi-porphyrin assemblies on the nano-scale compared to the internal modification of ODNs. To that end, we prepared four different strands (**ODN-4** to **ODN-7**) with a terminal **P-4** using amide coupling as described above; amide coupling was chosen due to the easier accessibility of the porphyrin and the costs associated with the BCN modifier. The strands are designed to assemble with appropriate complementary strands **ODN-4'** to **ODN-7'** to form an array of porphyrins, where the staple strands contain a complementary sequence to part of one porphyrin-ODN at the 5'-end, and a complementary sequence to a second porphyrin-ODN at the 3'-end.

```
ODN-4    5'-P ATG CTA AGG T-T-C GAT CGA TGC TAC GGT
ODN-4'     3`-TAC GAT TCG A-T-G GTC GCT CTC CTA AGT
ODN-5    5`-P ATT CTA ACT T-T-C CAG CGA GAG GAT TCA
ODN-5'     3`-TAA GAT TGA A-T-A AAC GAA TGT CCG CGT
ODN-6    5`-P GGT ACT CAT A-T-T TTG CTT-ACA-GGC-GCA
ODN-6'     3`-CCA TGA GTA T-T-A GGT CAG TTA AAG TAC
ODN-7    5`-P TTA GTA AGG T-T-T CCA GTC AAT TTC ATG
ODN-7'     3`-AAT CAT TCC A-T-G GTA TCA TCG CCT ATA
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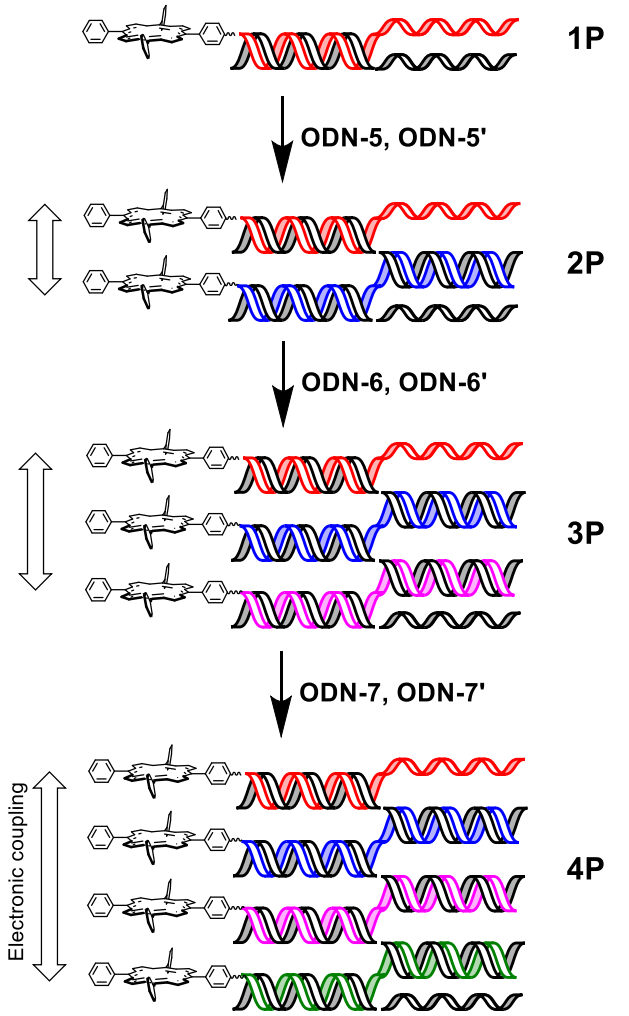

Figure 2. Design principle of the DNA templated supramolecular assembly and schematic depiction of multiporphyrin arrays. The colours of the sequences correspond to the schematic representation on the bottom, and the central T-base acts as a hinge in the cross-overs. The assembly of **ODN-4** / **ODN-4'** is denoted as **1P**, **ODN-4** / **ODN-4'** / **ODN-5** / **ODN-5'** as **2P** etc.

The formation of assemblies can partially be observed using native gel electrophoresis (PAGE; **Fig. 3A**). It appears that only the formation of the dimer is clearly visible, while the three and four porphyrin assemblies might show as very faint bands. Thus, under the conditions of PAGE the higher assemblies are either not stable, or they may not form properly in solution due to the crowding around the junctions. A control with unmodifed ODNs showed the full assembly (see ESI).

However, using absorption and emission spectroscopy suggests the assembly of the full array. To better see if mixing of the porphyrin-DNA strands has any impact on the electronics of the porphyrin, we compared the molar absorption coefficients

(ε) as shown in **Fig. 3B** and **3C**. In the first experiment, we mixed the individual strands but omitting the staple strands, i.e. ODN-4, ODN-4 + ODN-5 etc. We refer to this as system 1 and is in essence just increasing the concentration of porphyrin-ODN from 5 µM to 20 µM; we did not observe any impact of the ODN sequence on the spectroscopic properties of the porphyrins. The second experiment, referred to as system 2, consists of mixing the porphyrin-ODN with the complementary staple strands as shown in **Fig. 2**.

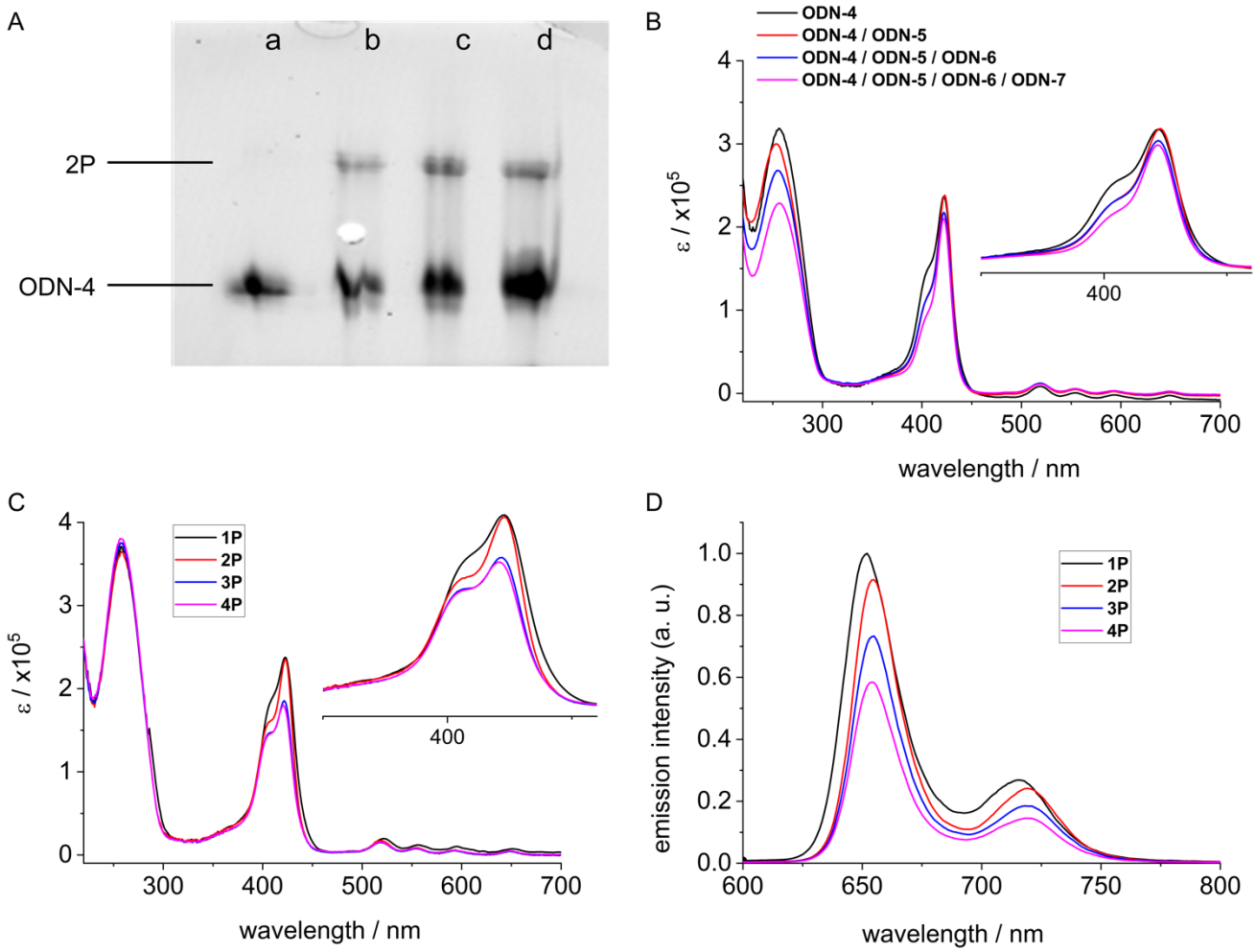

Figure 3. Analysis of the supramolecular porphyrin-DNA assemblies; for annotation see caption to **Figure 2**. **A**: Native PAGE analysis of the hybridised assemblies; only dimers are visible whereas higher assemblies seem to be disrupted. Lanes: a: **ODN-4**; b: **2P;** c: **3P**; d: **4P**. **B**: UV-vis spectra of mixtures of the ssODNs indicating hydrophobic interactions of the porphyrins in solution. **C**: UV-vis and **D**: steady state fluorescence (λ_{ex} = 417 nm) spectra of the assemblies with increasing number of hybridised strands. The emission intensity was normalised to the system with the highest intensity (**1P**). The insets in **B** and **C** show an expansion of the Soret-band absorbances. Spectra were recorded in PBS buffer (*p*H 7.4) at a total assembly concentration of 5 µM and using 0.1 cm quartz cuvettes.

In the UV-vis spectra, system 1 shows a gradual increase of intensity when adding the strands consecutively, as expected, but ε is overall not much affected for the absorbance maximum (**Fig. 3B**). The strand **ODN-4** shows a relatively broad and partially split spectrum in the Soret-band region at ~420 nm, which arises from intramolecular interactions of the porphyrins.[46,61,69,70] Addition of more strands leads to a sharpening of the Soret-band absorption which suggests that the interactions between the porphyrins are reduced, despite the higher concentration. This could be attributed to the presence of

the ODN single strands, though we do not have a clear picture on the potential interactions. For system 2, the ODN strands are mixed and annealed through heating to 90 °C and slow cooling to room temperature (0.1 °C / min). The assembled strands show much more pronounced split Soret band signatures, which is consistent with the close alignment of all porphyrins involved and leading to an electronic coupling between the chromophores; overall, the ε values decrease more compared to system 1. We take this as a strong argument that the system is assembled, at least to a very large percentage.

According to the point-dipole approximation of the exciton coupling model, the split signals arise from the interactions of the lower energy B_x and higher energy B_y dipoles.[71] The strand **ODN-4** itself shows a λ_{max} at 417 nm, whereas the split Soret absorbances can be found at $\lambda = 409$ (B_y) and at $\lambda = 424$ nm (B_x), which were obtained from *Gaussian* peak deconvolution (see ESI for graphs and Table 1 below for calculated data). The shifts indicate that the B_x are coupled as a *J*-aggregate to give rise to the red-shifted Soret band, and the By are coupled as a *H*-aggregate to give rise to the blue-shifted Soret band, respectively. All porphyrin assemblies show this splitting of the Soret band absorption. It should be noted that a third broad peak is generally present which manifests itself as a high energy shoulder in the Soret band, which we tentatively attribute to the absorption due to the phenyl substituents on the porphyrins.

Following literature procedures,[72,73] the overall transition dipole moments and the average centre−to−centre distances for the porphyrins can be estimated from the area of the absorbance (integral) and from the energies of the split signals, respectively (see ESI for details). From the absorbance of **ODN-4**, the dipole moment was determined to be $\mu = 11.7$ D which compares well to the values that we determined earlier for diphenyl-porphyrin-ODNs (11.1 D and 9.6 D).[72] The other porphyrin-ODNs gave similar values (not shown). Comparing the mixture of the four porphyrin-ODNs in system 1 with the **4P** assembly, the overall dipole moment for the former was determined to be 9.8 D, whereas for **4P** it was 10.5 D. Overall it seems that the transition dipole moments are not hugely affected by the presence of either single or double stranded ODNs, or whether they are self-assembled or loosely associated, though they tend to get smaller by adding more porphyrins to the assemblies.

Interesting is to compare the areas of the deconvoluted peaks: in all systems, the higher energy peak (B_y) contributes more to the absorbance than the lower energy peak (B_x) (Table 1, ESI). In the loosely associated porphyrins of system 1, the ratio of the two peaks is highest for **ODN-4** at 3.3:1, and lowest for the mixture of all four porphyrin ODNs at 1.7:1, whereas in the assemblies of system 2 the ratios vary from 3.2:1 to 5.1:1. The assembly **1P**, which is basically **ODN-4** with its partially complementary ODN strand, shows the highest ratio, which drops for the assembly **2P** and then moving back up to ~5:1 for **4P**. This behaviour could be interpreted in a way that the porphyrins generally show more *H*-aggregate feature, which is strengthened by the presence of the complementary strands. In other words, organising the porphyrins through DNA self-assembly has a great impact in the way that the porphyrins interact with each other.

The centre–to–centre distances, which were calculated from the energy difference of B_x and B_y together with the transition dipole moment, does not show a very large difference between the various system. This is in a way surprising given the differences in aggregation behaviour according to the deconvoluted Soret absorbance. The distances are around 11 to 12 Å and tend to become slightly shorter with more porphyrins present in the systems. This could be due to either increased concentration in system 1, or a re-orientation of the porphyrins to form an assembly with a higher proportion of a closer contact *H*-aggregate in system 2. Overall, these distances are larger than in porphyrin-DNA systems where multiple porphyrins are attached directly to the same DNA strand and located next to each other (6.7 to 7.4 Å),[72] or where they are in close contact through intermolecular stacking (6.8 to 8.9 Å).[46] The additional linkers in the ODNs used

here compared to previous direct attachment of the porphyrins to a nucleobase therefore does not force the porphyrins into a close-contact but allows for more structural flexibility in the assemblies.

Table 1. Electronic and geometrical values determined from the absorbances of the porphyrin systems.

Porphyrin system	Peak maxima[a] B_x, B_y / cm^{-1}	Energy split[b] $\Delta\bar{\nu}$/ cm^{-1}	Peak area ratio B_y / B_x	Transition dipole moment / D	Distance / Å
ODN-4	23598, 24351	753	3.3	11.7	12.2
ODN-4 / ODN-5 / ODN-6 / ODN-7	23642, 24351	709	1.7	9.8	11.1
1P	23494, 24234	740	5.1	12.1	12.6
2P	23564, 24416	852	3.2	11.5	11.6
3P	23582, 24433	851	4.4	10.7	11.0
4P	23608, 24431	823	4.8	10.5	11.1

a: *Gaussian* deconvoluted main peaks of the Soret band; b: energy difference of B_x and B_y.

The self-assembled DNA system is very crowded around the junctions; thus, it can be assumed that the longer distance is a result of hydrophobic porphyrin interactions and electrostatic repulsion of the DNA backbones. The reduction in steady state emission intensity is, however, consistent with the electronic interaction between porphyrins on DNA as observed previously, thus supporting the formation of a supramolecular assembly. Whether the DNA strands are fully hybridised, or in part loosely connected *via* the porphyrin interactions, remains elusive at this point, but mixing the staple strands with the porphyrin-ODNs clearly has an impact on the overall structure as shown through the difference in electronic coupling, highlighting that the hybridisation should be considered as the driving force for this.

CONCLUSIONS

In summary, we have shown that different porphyrins can be attached to ODNs post-synthetically, either through amide coupling or using click chemistry. The ODNs were assembled in a programmed manner to produce porphyrin arrays, and we have demonstrated this by using four different template strands. The obtained tetra-porphyrin array shows electronic coupling between the units, indicative of formation of a π-stacked array with close contact. Since this methodology can easily be extended to form longer arrays, it provides an alternative way to form porphyrin wires, making use of the unique recognition properties of DNA and applying the concepts of DNA based bio-nanotechnology to form programmed functional entities. However, as mentioned above the full and intact assembly through hybridisation will still need to be confirmed.

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REFERENCES

1. Gray V, Moth-Poulsen K, Albinsson B and Abrahamsson M. *Coord. Chem. Rev.* 2018; **362**: 54-71.
2. Rousseaux SAL, Gong JQ, Haver R, Odell B, Claridge TDW, Herz LM and Anderson HL. *J. Am. Chem. Soc.* 2015; **137**: 12713-12718.
3. D'Urso A, Fragala ME and Purrello R. *Chem. Commun.* 2012; **48**: 8165-8176.
4. Nakamura Y, Aratani N and Osuka A. *Chem. Soc. Rev.* 2007; **36**: 831-845.
5. Balaban TS, Berova N, Drain CM, Hauschild R, Huang XF, Kalt H, Lebedkin S, Lehn JM, Nifaitis F, Pescitelli G, Prokhorenko VI, Riedel G, Smeureanu G and Zeller J. *Chem.-Eur. J.* 2007; **13**: 8411-8427.
6. Rioz-Martinez A and Roelfes G. *Curr. Opin. Chem. Biol.* 2015; **25**: 80-87.
7. Zang Y, Lei JP, Ling PH and Ju HX. *Anal. Chem.* 2015; **87**: 5430-5436.
8. Occhiuto IG, Samperi M, Trapani M, De Luca G, Romeo A, Pasternack RF and Scolaro LM. *J. Inorg. Biochem.* 2015; **153**: 361-366.
9. Ning JH, Wang YF, Wu Q, Zhang XF, Lin XF and Zhao HB. *RSC Adv.* 2015; **5**: 21153-21160.
10. Van Raden JM, Alexandropoulos DI, Slota M, Sopp S, Matsuno T, Thompson AL, Isobe H, Anderson HL and Bogani L. *J. Am. Chem. Soc.* 2022; **144**: 8693-8706.
11. Jing HY, Rong J, Taniguchi M and Lindsey JS. *Coord. Chem. Rev.* 2022; **456**: 202.
12. Tanaka T and Osuka A. *Chem.-Eur. J.* 2018; **24**: 17188-17200.
13. Balaz M, Tannir S and Varga K. *Coord. Chem. Rev.* 2017; **349**: 66-83.
14. Ensslen P and Wagenknecht HA. *Acc. Chem. Res.* 2015; **48**: 2724-2733.
15. Zhang N, Chu XZ, Fathalla M and Jayawickramarajah J. *Langmuir* 2013; **29**: 10796-10806.
16. Varghese R and Wagenknecht HA. *Chem.-Eur. J.* 2010; **16**: 9040-9046.
17. Stulz E. *Acc. Chem. Res.* 2017; **50**: 823-831.
18. Govindaraju T and Ariga K. *Molecular Architectonics and Nanoarchitectonics*; Springer Nature Singapore Pte Ltd: Singapore, 2022
19. Varghese R and Wagenknecht HA. *Chem. Commun.* 2009; **19**: 2615-2624.
20. Pathak P, Zarandi MA, Zhou X and Jayawickramarajah J. *Front. Chem.* 2021; **9**: 30.
21. Hofsass R, Ensslen P and Wagenknecht HA. *Chem. Commun.* 2019; **55**: 1330-1333.
22. Sargsyan G, Leonard BM, Kubelka J and Balaz M. *Chem.-Eur. J.* 2014; **20**: 1878-1892.
23. Balaz M, Bitsch-Jensen K, Mammana A, Ellestad GA, Nakanishi K and Berova N. *Pure Appl. Chem.* 2007; **79**: 801-809.
24. Malinovskii VL, Wenger D and Häner R. *Chem. Soc. Rev.* 2010; **39**: 410-422.
25. Xiang YG, Zhang QY, Li ZB and Chen H. *Mater. Sci. Eng. C-Mater. Biol. Appl.* 2017; **70**: 1156-1162.
26. Murashima T, Hayata K, Saiki Y, Matsui J, Miyoshi D, Yamada T, Miyazawa T and Sugimoto N. *Tetrahedron Lett.* 2007; **48**: 8514-8517.

27. Berlin K, Jain RK, Simon MD and Richert C. *J. Org. Chem.* 1998; **63**: 1527-1535.
28. Vybornyi M, Nussbaumer AL, Langenegger SM and Häner R. *Bioconjugate Chem.* 2014; **25**: 1785-1793.
29. Fendt LA, Bouamaied I, Thoni S, Amiot N and Stulz E. *J. Am. Chem. Soc.* 2007; **129**: 15319-15329.
30. Bouamaied I, Fendt LA, Häussinger D, Wiesner M, Thöni S, Amiot N and Stulz E. *Nucleos Nucleot Nucl* 2007; **26**: 1533-1538.
31. Bouamaied I, Fendt LA, Wiesner M, Häussinger D, Amiot N, Thöni S and Stulz E. *Pure Appl. Chem.* 2006; **78**: 2003-2014.
32. Nguyen T, Brewer A and Stulz E. *Angew. Chem. Int. Ed.* 2009; **48**: 1974-1977.
33. Mammana A, Pescitelli G, Asakawa T, Jockusch S, Petrovic AG, Monaco RR, Purrello R, Turro NJ, Nakanishi K, Ellestad GA, Balaz M and Berova N. *Chem.-Eur. J.* 2009; **15**: 11853-11866.
34. Sargsyan G and Balaz M. *Org. Biomol. Chem.* 2012; **10**: 5533-5540.
35. Endo M, Fujitsuka M and Majima T. *J. Org. Chem.* 2008; **73**: 1106-1112.
36. Malecka K, Kaur B, Cristaldi DA, Chay CS, Mames I, Radecka H, Radecki J and Stulz E. *Bioelectrochem.* 2021; **138**: 107723.
37. Kaur B, Malecka K, Cristaldi DA, Chay CS, Mames I, Radecka H, Radecki J and Stulz E. *Chem. Commun.* 2018; **54**: 11108-11111.
38. Pursey JP, Chen Y, Stulz E, Park MK and Kongsuphol P. *Sensor Actuat B-Chem* 2017; **251**: 34-39.
39. Takada T, Iwaki T, Nakamura M and Yamana K. *Chem.-Eur. J.* 2017; **23**: 18258-18263.
40. Börjesson K, Woller JG, Parsa E, Martensson J and Albinsson B. *Chem. Commun.* 2012; **48**: 1793-1795.
41. Borjesson K, Lundberg EP, Woller JG, Norden B and Albinsson B. *Angew. Chem. Int. Ed.* 2011; **50**: 8312-8315.
42. Borjesson K, Wiberg J, El-Sagheer AH, Ljungdahl T, Martensson J, Brown T, Norden B and Albinsson B. *ACS Nano* 2010; **4**: 5037-5046.
43. Börjesson K, Tumpane J, Ljungdahl T, Wilhelmsson LM, Norden B, Brown T, Martensson J and Albinsson B. *J. Am. Chem. Soc.* 2009; **131**: 2831-2839.
44. Llamas EM, Tome JPC, Rodrigues JMM, Torres T and Madder A. *Org. Biomol. Chem.* 2017; **15**: 5402-5409.
45. Burns JR, Gopfrich K, Wood JW, Thacker VV, Stulz E, Keyser UF and Howorka S. *Angew. Chem. Int. Ed.* 2013; **52**: 12069-12072.
46. Nguyen T, Hakansson P, Edge R, Collison D, Goodman BA, Burns JR and Stulz E. *New J. Chem.* 2014; **38**: 5254-5259.
47. Mammana A, Asakawa T, Bitsch-Jensen K, Wolfe A, Chaturantabut S, Otani Y, Li XX, Li ZM, Nakanishi K, Balaz M, Ellestad GA and Berova N. *Bioorg. Med. Chem.* 2008; **16**: 6544-6551.
48. Wellner C and Wagenknecht HA. *Org. Lett.* 2014; **16**: 1692-1695.
49. Endo M, Fujitsuka M and Majima T. *Tetrahedron* 2008; **64**: 1839-1846.
50. Ladomenou K, Nikolaou V, Charalambidis G and Coutsolelos AG. *Coord. Chem. Rev.* 2016; **306**: 1-42.
51. Sau SP and Hrdlicka PJ. *J. Org. Chem.* 2012; **77**: 5-16.
52. Xiong H and Seela F. *J. Org. Chem.* 2011; **76**: 5584-5597.
53. Jacobsen MF, Ravnsbaek JB and Gothelf KV. *Org. Biomol. Chem.* 2010; **8**: 50-52.
54. El-Sagheer AH and Brown T. *Chem. Soc. Rev.* 2010; **39**: 1388-1405.
55. Clave G, Chatelain G, Filoramo A, Gasparutto D, Saint-Pierre C, Le Cam E, Pietrement O, Guerineau V and Campidelli S. *Org. Biomol. Chem.* 2014; **12**: 2778-2783.

56. Chatelain G, Clave G, Saint-Pierre C, Gasparutto D and Campidelli S. *Org. Biomol. Chem.* 2017; **15**: 6257-6263.
57. Stephenson AWI, Partridge AC and Filichev VV. *Chem.-Eur. J.* 2011; **17**: 6227-6238.
58. Stephenson AWI, Bomholt N, Partridge AC and Filichev VV. *ChemBioChem* 2010; **11**: 1833-1839.
59. Anderson NT, Dinolfo PH and Wang X. *J. Mater. Chem. C* 2018; **6**: 2452-2459.
60. Kosman J, Stanislawska A, Gluszynska A and Juskowiak B. *Int. J. Biol. Macromol.* 2017; **101**: 799-804.
61. Brewer A, Siligardi G, Neylon C and Stulz E. *Org. Biomol. Chem.* 2011; **9**: 777-782.
62. Nikolaou V, Angaridis PA, Charalambidis G, Sharma GD and Coutsolelos AG. *Dalton Trans.* 2015; **44**: 1734-1747.
63. Zervaki GE, Papastamatakis E, Angaridis PA, Nikolaou V, Singh M, Kurchania R, Kitsopoulos TN, Sharma GD and Coutsolelos AG. *Eur. J. Inorg. Chem.* 2014; **2014**: 1020-1033.
64. baseclick. "ClickTech Oligo Link Kit." https://www.baseclick.eu/product/clicktech-oligo-link-kit/. Accessed 05-01-2023
65. Stephenson AWI. *Ph.D. thesis*; Massey University: Supramolecular helical arrangement of porphyrins along DNA, 2010.
66. Geci I, Filichev VV and Pedersen EB. *Chem.-Eur. J.* 2007; **13**: 6379-6386.
67. Gangemi CMA, Randazzo R, Gaeta M, Fortuna CG, Fragala ME, Purrello R and D'Urso A. *J. Porphyrins Phthalocyanines* 2020; **24**: 802-808.
68. Fanti C, Monti D, La Monica L, Ceccacci F, Mancini G and Paolesse R. *J. Porphyrins Phthalocyanines* 2003; **7**: 112-119.
69. Singleton DG, Hussain R, Siligardi G, Kumar P, Hrdlicka PJ, Berova N and Stulz E. *Org. Biomol. Chem.* 2016; **14**: 149-157.
70. Zanetti-Polzi L, Amadei A, Djemili R, Durot S, Schoepff L, Heitz V, Ventura B and Daidone I. *J. Phys. Chem. C* 2019; **123**: 13094-13103.
71. Hwang IW, Park M, Ahn TK, Yoon ZS, Ko DM, Kim D, Ito F, Ishibashi Y, Khan SR, Nagasawa Y, Miyasaka H, Keda C, Takahashi R, Ogawa K, Satake A and Kobuke Y. *Chem.-Eur. J.* 2005; **11**: 3753-3761.
72. Bouamaied I, Nguyen T, Rühl T and Stulz E. *Org. Biomol. Chem.* 2008; **6**: 3888-3891.
73. Anderson HL. *Inorg. Chem.* 1994; **33**: 972-981.

Post-synthetic DNA modification with porphyrins for DNA templated supramolecular assemblies

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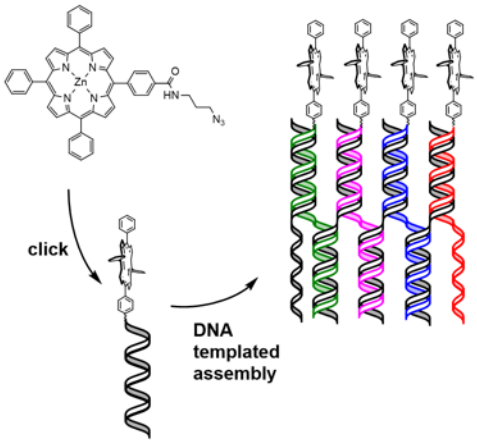
Post synthetic modification of DNA with porphyrins is very efficient using copper free “click chemistry”. The porphyrin-DNA strands can be transformed into multiporphyrin arrays through a DNA templated assembly strategy, which opens an alternative route to systems for electronic applications.



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