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Bioorganic Hybrid Nanomaterials in Optics, Electronics and Sensing

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Abstract

DNA has become an attractive scaffold for supramolecular systems, including chromophore arrays for optical and electronic applications. Here we will present our research in using DNA as a construction material for optoelectronic systems, which are mainly based on the formation of helical porphyrin chromophore arrays, obtained using rational synthesis and bio-templating. The porphyrin is also very sensitive for electrochemical detection of DNA, for which we are creating both microelectrodes and microfluidic systems.

1. Introduction

DNA is well-known as bearer of the genetic code. Since its structure elucidation nearly seven decades ago by Watson, Crick, Wilkins, and Franklin, much has been learned about its detailed structure, function, and genetic coding. The development of automated solid-phase synthesis, and with it the availability of synthetic DNA with any desired sequence in lengths of up to hundreds of bases in the best case, has contributed much to the advancement of the field of DNA research. In addition, classic organic synthesis has allowed introduction of a very large number of modifications in the DNA in a sequence specific manner. In recent years DNA has become a very attractive scaffold in supramolecular chemistry, where DNA is taken out of its biological role and serves to assemble novel functional structures with nanometer precision. The attachment of functionalities to DNA has led to the creation of supramolecular systems with applications in light harvesting, energy and electron transfer, sensing, and catalysis. Functional DNA is clearly having a significant impact in the field of bioinspired nanosystems.[1]

Of particular interest is the use of porphyrins: they are excellent functional groups due to their electronic properties that can be tailored through chemical modifications of the aromatic core, or through insertion of almost any metal of the periodic table into the central cavity. Here we will present our porphyrin-DNA based systems which have shown to be versatile for the creation of photonic wires through formation of chiral helical chromophore stacks in a programmable manner. On the other hand, the electrochemical properties of the porphyrin allow for applying the system in the realisation of highly sensitive detectors for both viral and cancer related DNA.

2. Results

Supramolecular photonic wires

To create multiporphyrin arrays using DNA as an underlying scaffold, where the porphyrins are held rigidly and in a predictable way on the DNA, new strategies had to be developed, and we found that the use of *Sonogashira* coupling between 5-iodo-deoxyuridine (5-iodo-dU) and alkyne porphyrins is most versatile to synthesise building blocks for programmed insertion into DNA: the porphyrin is attached to the nucleobase and will protrude from the DNA into the major groove. In this way, we have created porphyrin-DNA arrays that incorporate a large number of chromophores which show strong excitonic interactions between the units, and efficient intramolecular energy TRANSFER between different porphyrin units. This was shown through optical spectroscopy including absorbance, emission and circular dichroism spectroscopy (Figure 1).[2]

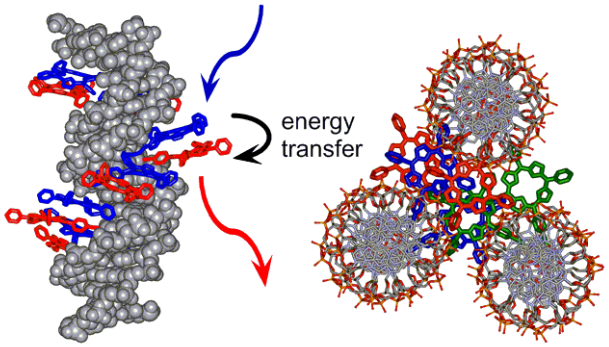

Fig. 1. Helical arrangement of porphyrins along a DNA template for induced energy transfer (left), and bundling of porphyrin-DNA based on hydrophobic interactions (right).

It should be noted that the hydrophobic nature of the porphyrin extends the interactions beyond the underlying DNA strand, and formation of intermolecular stacks have become evident. This leads to the formation of discrete bundles where porphyrins show strong electronic coupling with neighbouring porphyrin-DNA strands, as shown by CD, EPR and small angle X-Ray spectroscopy (SAXS). This can be prevented by either using more water soluble porphyrin derivatives, or by using better bio-templating. We are pursuing the use of DNA binding proteins to form long-range photonic wires, where energy can be transmitted over a range of >15 nm.

Trans-membrane ionic current generation

The hydrophobic nature of the porphyrin allows to insert modified DNA into lipid bilayers, mimicking the membrane of cells. We have attached porphyrins at strategically suitable places in DNA origami systems to embed a six helical DNA bundle in a lipid bilayer. This creates an artificial nano-pore with a central hole of around 2 nm in width. Ionic current can be generated by applying a voltage across the membrane, and

the flow of ions can be measured as a function of the transmembrane pore size (Figure 2). Two porphyrins are sufficient in embedding the large DNA structure, whereas a large number of small modifiers would be required to achieve the same result. The porphyrin also offers an optical handle to detect the membrane insertion, thus acting as a modifier with dual functionality. Interestingly, a simple DNA duplex incorporating six porphyrins also inserts efficiently into the membrane and creates a transmembrane ion flow to induce a current. With this we have made the smallest possible DNA based nanopore in lipid bilayers.[3] Both systems are now being evaluated in cancer therapy.

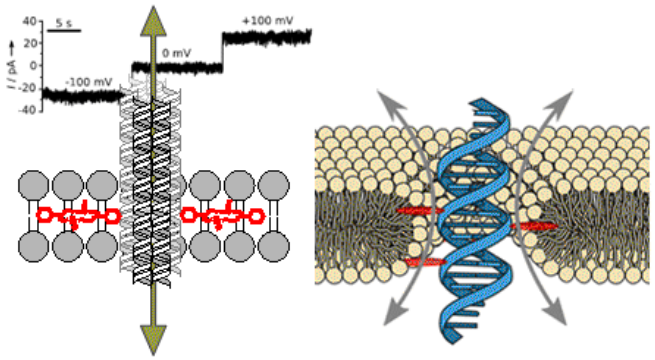

Fig. 2. DNA origami spanning lipid bilayer to create nanopore and inducing ionic current (left); smallest possible DNA based membrane nanopore including six porphyrins in a DNA duplex (right).

Electrochemical sensors

Porphyrins are not only optically active, but have a rich electrochemistry which depends strongly on the structure, central metal and micro-environment. We have used both free-base and cobalt metallated porphyrins for the generation of genosensors on gold electrodes. These can be embedded in microfluidic devices to generate multiplexing sensors.

First, we used cobalt porpyrin-DNA to create highly sensitive geno-sensors (Fig. 4). The porphyrin is located close to the electrode surface, and we found that upon duplex formation the ionic current is greatly diminished. This is explained by placing the porphyrin into the more hydrophobic major groove of the dsDNA and giving it limited access to the electrolyte, compared to the exposed single strand arrangement. The mechanism is distinctively different to other distance based "signal on-off" systems. The selective detection of complementary strands including single-nucleotide polymorphism was demonstrated, and it was calculated that as few as 1000 DNA molecules can be measured. In this way, an Avian Influenza Virus (H5N1) based DNA sequence was detected at femtomolar levels from competing non-complementary sequences.[4] By inclusion of nanoparticles into the system, the sensitivity can be improved substantially, and DNA can easily be detected at the attomolar level with a limit of detection of ~10^{-18} M.

Second, we have used the free-base porphyrin in a molecular beacon arrangement to detect DNA markers in urine, which are diagnostic for bladder cancer. The working principle is very much the same as in the above system, and we observed a substantial increase in current upon duplex formation. Here the hairpin is a "signal-off" state because is in effect in a duplex environment, whereas when hybridised with the target the porphyrin is attached to a single strand overhang and is therefore in the "signal-on" state, in line with our previous observations. By embedding the molecular beacon in a microfluidic array consisting of 20 micro-electrodes, simultaneous multiplexing of three distinct bladder cancer marker genes could be achieved with a detection limit of around 200 fM, which is well below the clinically relevant amount of DNA found in urine.[5] Therefore, this system may find application in point-of-care diagnostics for cancer, but could equally be applied to the detection of bacterial or viral infections.

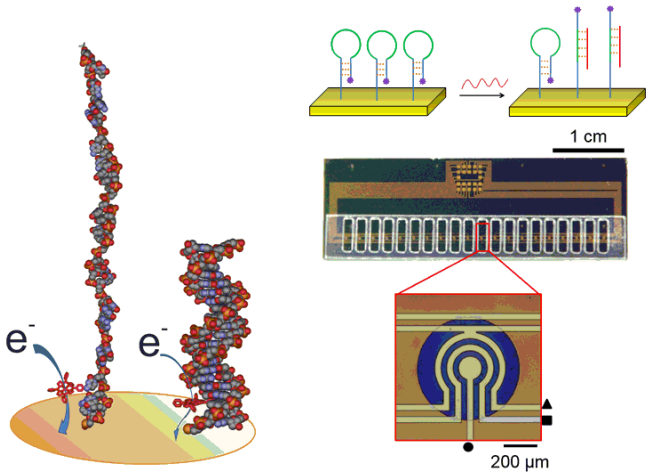

Fig. 3. Electrochemical genosensor detecting avian flu virus DNA (left), and multiplexing microfluidic DNA detection for cancer and bacterial / viral DNA markers (right).

3. Conclusions

We have shown that modified DNA provides an extremely versatile building block in all aspects of science, ranging from optics over electronics to sensing and diagnostics. In particular porphyrins, which have a high diversity in optical and electrochemical properties, are highly attractive to create systems with tailor made functionality.

Acknowledgements

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