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Development of donor–acceptor modified DNA hairpins for the investigation of charge hopping kinetics in DNA

Nicole Rahe, Cornelia Rinn and Thomas Carell*
Department of Chemistry, Philipps-University, D-35032 Marburg, Germany.
E-mail: carell@staff.uni-marburg.de; Fax: 49 (0)6421 282 2189; Tel: 49 (0)6421 282 1535

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Investigation of hole or excess electron hopping in DNA is mostly performed based on yield studies, in which an injector modified DNA duplex is irradiated to continuously inject either holes or electrons into the duplex. Observed is a chemical reaction of a "probe" molecule, which can be either one of the two purine bases or a different trap molecule positioned at various distances. The next step in the field will be the direct time resolution of the hole or electron transfer kinetics in DNA. Herein we describe the development of defined donor–DNA–acceptor systems, with properties that may allow time resolved electron and hole transfer studies in stably folded DNA structures.

The transfer of holes and of electrons through DNA is an actual research topic in contemporary bioorganic research.[1–4] The ability to synthesize defined DNA-based model systems recently enabled the elucidation that holes and electrons travel through DNA over significant distances by hopping. It is today clear that holes hop *via* guanines and to a lesser extend also *via* adenines through the base stack.[5] Early irradiation studies[6,7] suggest that electrons in turn use thymines and cytosines as hopping steps.[8–10] We introduced recently a defined model system, which allows the study of both, hole and electron transfer in DNA.[11–14] The model contains a flavin, which upon irradiation donates holes into the DNA duplex. Reduction of the flavin molecule, however, converts it into a strong electron donor able to photo-reduce all nucleobases (electron injection). Whereas hole transfer can be detected based on the reaction of the intermediate A- and G-radical cations with water,[15] giving rise to detectable DNA lesions,[16,17] uncovering of the electron transfer event required the additional presence of an acceptor. This translates electron capture into a chemical or spectroscopic signal. The next step in the field will be time resolved experiments[18–20] needed to learn about the charge transfer kinetics in DNA.[4,21–25] Herein, we describe the development of a model system for the time resolved studies.

Similar to Lewis *et al.*[25] we chose DNA hairpins as the basis for the new model systems because hairpins feature high and concentration independent melting points. The hairpins (**1–6**) designed for the time resolved studies are depicted in Fig. 1. For charge injection they possess a flavin cap. We planned to investigate hole transfer using time resolved fluorescence spectroscopy because hole injection into A and G bases gives a strong fluorescence quenching of the flavin chromophore. Analysis of the excess electron transfer kinetics in contrast is planned with time resolved absorption spectroscopy, which demands the additional presence of an electron acceptor giving a readily detectable absorption change after single electron reduction. To achieve excess electron detection we chose to incorporate either a naphthalene diimide or a perylene diimide into the hairpin. These molecules are known to give a strong absorption increase at 474 nm (naphthalene) and 700 nm (perylene) upon single electron reduction.[26]

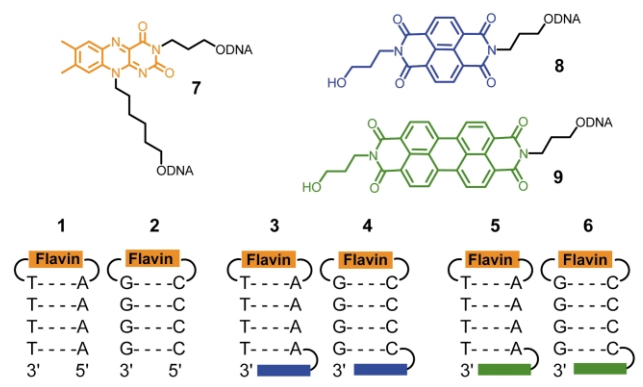
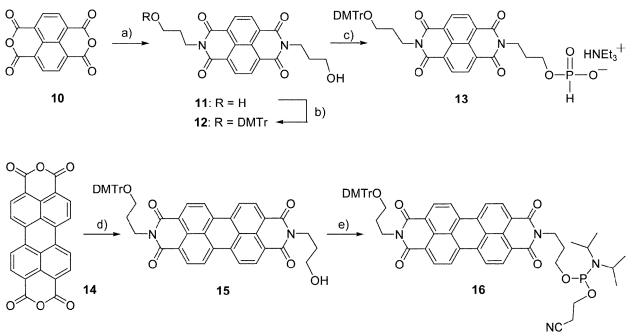


Fig. 1 Depiction of the flavin-**7** capped, acceptor-**8** and -**9** modified DNA hairpins **1–6** constructed for kinetic studies of hole and electron transfer through DNA.

The synthesis of the flavin **7** was recently reported.[11,12] Synthesis of the naphthalene diimide *H*-phosphonate **8**, and of the perylene diimide phosphoramidite **9**, needed for the automated DNA synthesis, is depicted in Scheme 1.[27,28] The naphthalene dianhydride **10** was first activated with zinc acetate and then reacted with 1-amino-propan-3-ol to give the symmetrical naphthalene diimide **11**. Reaction of **11** with 1 eq. of DMT–Cl and conversion of the mono-protected naphthalene diimide **12** into the *H*-phosphonate allowed preparation of **13** in just 3 steps in an overall yield of 18%.

Activation of the perylene dianhydride **14** with zinc acetate was followed by reaction with a 1 : 1 mixture of 1-amino-propan-3-ol and of the DMT-protected 1-amino-propan-3-ol. This gave a mixture of products out of which the perylene diimide **15** was isolated by flash chromatography on silica. This procedure allowed circumvention of the extremely poor solubility of the perylene compounds, which was a major obstacle on the way to the flavin and perylene modified DNA strands. **15** was converted into the phosphoramidite **16**.

The synthesis of the DNA hairpins **1–6** required a complex *H*-phosphonate–phosphoramidite DNA synthesis protocol.[29–32] DNA synthesis started with phosphoramidite chemistry. Incorporation of the flavin was achieved using an *H*-phosphonate building block. This required simultaneous pumping of the flavin *H*-phosphonate and of 1-adamantane carbonyl chloride as the activator. Subsequent oxidation converted the *H*-phospho-



Scheme 1 Synthesis of the acceptor building blocks used to construct flavin–DNA–acceptor modified DNA hairpins. a) $Zn(OAc)_2 \cdot 2H_2O$, py., $NH_2(CH_2)_3OH$, reflux, 65%. b) DMTCl, py. 38%. c) i: PCl_3, triazole, 4-methylmorpholine, ii: TEAB, pH 7.8, 73%. d) $Zn(OAc)_2 \cdot 2H_2O$, py., 1 eq. $NH_2(CH_2)_3OH$, 1 eq. $NH_2(CH_2)_3ODMT$, reflux, 28%. e) CED–Cl, DIEA, CH_2Cl_2, 43%.

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natediester into the phosphate diester. The synthesis was continued using phosphoramidite chemistry. Incorporation of the perylene diimide was achieved using standard phosphoramidite chemistry in CH_2Cl_2. For the naphthalene diimide we used another *H*-phosphonate step at the end of the synthesis cycle. The DNA strands were finally again oxidized (0.2 M I_2 in MeCN with a: *N*-methylmorpholine and b: TEA) to convert any remaining P(III) into P(V). Final cleavage of all protection groups and release of the DNA from the solid support was performed with saturated NH_3–water in ethanol (3 : 1) at 55 °C over 15 h. All oligonucleotides were purified by reversed phase HPLC. UV-melting studies showed melting points of all hairpins above rt. Extending the stem of the hairpins by two additional base pairs would raise the melting point to immeasurably high values.

UV- and fluorescence studies show that the prepared hairpins possess all the properties required for the envisioned detailed short time spectroscopic studies. Fig. 2a shows the fluorescence of a flavin and of the two hairpins **1** and **2**. Clearly visible is the strong fluorescence reduction of the flavin in the hairpins **1** and **2** reflecting the light induced oxidation (hole injection) of the nucleobases G and A. In accord with the lower oxidation potential of the guanine base we detected a stronger fluorescence reduction in hairpin **2**. More interesting is the question if the hairpins **3–6** would allow time resolved absorption studies. Here selective reduction of the flavin in the presence of the acceptors is a prerequisite. This was achieved by photoreduction (Fig. 2b).

The naphthalene containing hairpins **3** and **4** possess two absorption bands around 380 nm and 450 nm (red curve) caused predominantly by the oxidized flavin and the naphthalene (380 nm). Irradiation of the hairpins **3** and **4** in the presence of ethylenediamine tetraacetic acid (EDTA) with white light allowed selective photoreduction of the flavin chromophore (blue curve) in the AT as well as GC rich hairpins along with the formation of a small amount of the naphthalene diimide monoanion. Addition of dithionite, in contrast, as a powerful reductance, gives rise immediately to two additional absorption maxima at 520 nm and 575 nm. These absorptions are caused by the doubly reduced naphthalene acceptor. All these bands are well separated from the flavin absorption, which is a prerequisite for short time spectroscopic studies. The UV-spectroscopic signature of the perylene hairpins – data not shown – reveal similar behaviour. Selective photoreduction of the flavin is possible in the AT hairpin **5**. In hairpin **6**, however, photoreduction fails due to an additional energy transfer from the flavin to the perylene, which further reduces the fluorescence life time of the flavin. In summary, we developed flavin–donor–acceptor DNA hairpins for short time spectroscopic studies. We can selectively photoreduce the flavin. The naphthalene acceptor anions possess well separated absorption bands perfectly suited for short time spectroscopic studies.

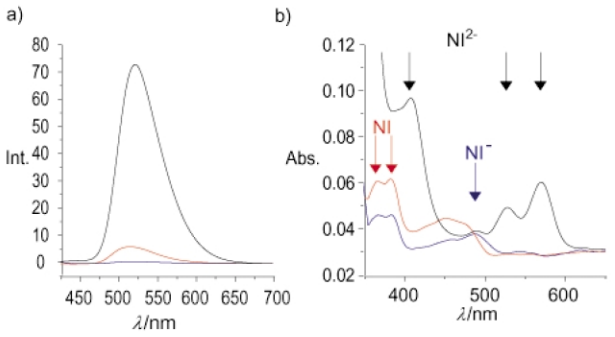

Fig. 2 a) Fluorescence spectra of the hairpins **1** (red) and **2** (blue) compared to flavin (black) in solution and b) UV spectra of the oxidized, the photoreduced and the fully dithionite reduced hairpin **3**. NI = naphthalene diimide, NI^- = monoanion, NI^{2-}: dianion.

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