Programmed assembly of organic radicals on DNA†

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Nitronyl nitroxide radical introduced to naphthyridine carbamate dimer is noncovalently bound to a CGG/CGG triad as an addressable position in DNA duplexes, leading to the programmed assembly of the radical molecules into an 11-mer duplex and a tandem repetitive array of double stranded DNA.

DNA molecules have been rediscovered as building blocks for constructing nanoscale objects by utilizing the sequence-specific hybridization of complementary strands.[1] DNA-based architectures can provide a well-defined programmable template with addressable and accessible base sequences,[2] exploring the possibility to assemble auxiliary molecules and functional groups on multi-dimensional DNA nanostructures.

Stable organic radicals have so far been exploited as spin probes in spin labeling chemistry to obtain the structural and dynamic insights into biologically important biomolecules.[3] Spin labeling for DNA by the covalent modification of nucleobases or the phosphate–sugar backbone has also been actively investigated.[4] To utilize the programmable DNA sequences, we have studied the non-covalent assembly of stable organic radicals on DNA arrays. The non-covalent assembly strategy has an advantage over the covalent introductions of stable organic radicals, as exploited in conventional spin labeling chemistry. DNA provides a variety of scaffolds for ligand bindings, *e.g.* a minor groove binding, an intercalation, and a major groove binding with a sequence specific manner. The combination of the modes of the ligand bindings and stable radical families such as nitroxides, and imino- or nitronyl nitroxides allows us to obtain a diversity of DNA-stable radical assembly with well-identified distances and the different spatial orientations of magnetic tensors, which can afford scalable electron spin-qubit systems for quantum computers/quantum information processing systems based on molecular spins.[5] The non-covalent strategy will provide rapid access to the assemblages of organic radicals for controlled functionalities. Here we report a DNA building block holding two stable organic radicals and its assemblage into a one-dimensional DNA array. The DNA unit described here has a sticky DNA overhang, which enables us to organize the unit into multi-dimensional DNA structures with the accessible complementary sequence.

We have synthesized a mismatch binding ligand holding nitronyl nitroxide (NCDNN). The parent mismatch binding ligand NCD (naphthyridine carbamate dimer) specifically binds to a G–G mismatch in a 5′-CGG-3′/5′-CGG-3′ triad in the ratio of NCD : triad = 2 : 1 and improved the thermodynamic stability of the duplex.[6] We anticipate that the binding of NCDNN to the CGG/CGG triad enables programmed assembling of the radical molecules at the designed position in a DNA structure (Fig. 1). NCDNN was synthesized by the reductive amination of p-formylphenyl nitronyl nitroxide with NCD. The thermal denaturation experiments of 11-mer DNA duplexes 5′-(CTAACXGAATG)-3′/5′-(CATTCYGTTAG)-3′, in which the symbols X and Y designate A, G, C, or T, were conducted to evaluate the thermodynamic stability of each duplex in the absence and presence of NCDNN. The presence of NCDNN led to a remarkable change of T_m for the duplex containing the G–G mismatch compared with the others, giving the difference of T_m of 13.5 °C (ΔT_m) (Table S1†). These results suggest the

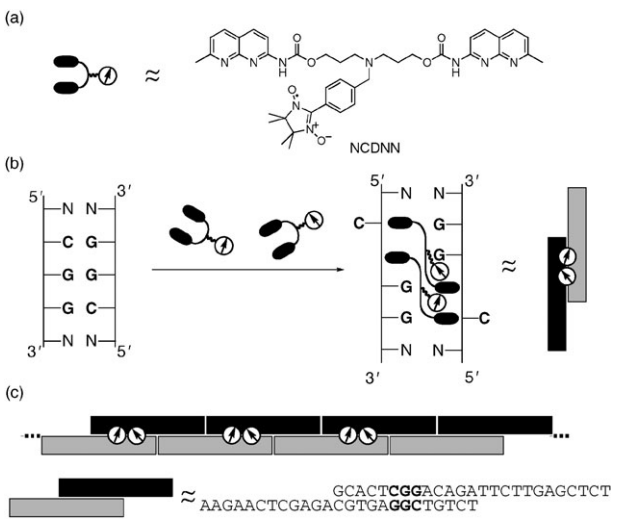

Fig. 1 (a) The structure of NCDNN. (b) The schematic representation of bindings of NCDNN to the CGG/CGG triad and the DNA unit embedded with the radicals. (c) The programmed assembly of the organic radicals on a tandem repetitive array of the double stranded DNA organized by sticky overhangs.

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selective binding of NCDNN to the mismatched duplex containing the CGG/CGG triad.

Titration experiments in circular dichroism (CD) spectra with the 11-mer DNA containing the CGG/CGG triad showed the increase of the induced CD bands with a negative and positive Cotton signal as the concentration of NCDNN increased (Fig. 2). The NMR structure of the complex composed of NCD and a duplex containing the CGG/CGG triad revealed that the two naphthyridine rings of two NCDs were stacked along the duplex with each other.[7] An exciton band originating from the stacking structure of the naphthyridine rings was in fact observed in the CD spectra. The exciton band has also been found around 328 nm. Job's plot analysis clearly showed the binding stoichiometry of NCDNN to the CGG/CGG triad to be 2 : 1 (Fig. S1†). The concentration dependence of molar ellipticity at 347 nm was fitted on the basis of an identical and independent two-site binding model to estimate a binding constant K_a.[8] The fit to the observed values gave K_a to be 1.6×10^5 M^{-1} with the 2 : 1 stoichiometry of NCDNN to DNA. These results indicate that (1) the binding mode of NCDNN to the CGG/CGG triad is similar to that of NCD and (2) two nitronyl nitroxide radicals bind to the triad in 10^5 affinity.

Fig. 3a and b show solution ESR spectra of NCDNN in the absence and presence of the 11-mer duplex containing the CGG/CGG triad, respectively. The spectrum of only NCDNN exhibited a typical hyperfine splitting pattern with equally separated five peaks attributed to the two nitrogen nuclei on the radical moiety. The spectrum of the radical solution containing the duplex showed a remarkable change featuring in a line broadening dependent on the m_I component of the nuclear spin I.[9] The broadening reflects a slower tumbling of the radical moieties compared with that of only the NCDNN. No electron spin forbidden transition with $\Delta m_s = \pm 2$ was observed for the duplex-containing sample in the frozen glass at 143 K. Both the observed spectra were simulated to evaluate a rotational correlation time τ_c assuming an isotropic rotational diffusion in a fast regime and only an $S = 1/2$ spin state. Due to the equilibrium of NCDNN binding to the CGG/CGG triad, the fraction of the bound

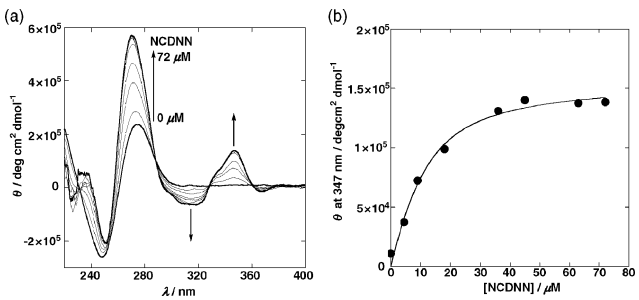

Fig. 2 (a) CD spectra for the titration of the DNA duplex (4.5 μM) in sodium cacodylate buffer (10 mM, pH 7.0) containing 100 mM NaCl and 10% (v/v) methanol with NCDNN. Key: [NCDNN] = 0, 4.5, 9, 18, 36, 45, 63, 72 μM. The arrows designate the change of a molar ellipticity with increased concentration of NCDNN. (b) Concentration dependence of the molar ellipticity at 347 nm. The closed circles and the solid line denote the observed values and the theoretical curve based on an identical and independent two-sites binding model.

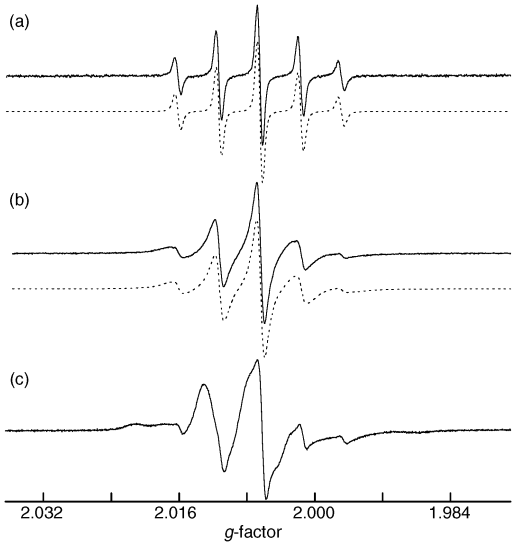

Fig. 3 ESR spectra of NCDNN (200 μM) in a buffer containing 100 mM NaCl, 10 mM sodium cacodylate (pH 7.0) and 10% (v/v) methanol in the (a) absence and (b) presence of the 11-mer DNA duplex (100 μM) at 297 K with microwave frequency ν_{MW} = 9.44601(7) GHz for (a) and 9.44482 (4) GHz for (b). The dashed lines represent simulated spectra. (c) A spectrum of NCDNN (200 μM) in a sodium cacodylate buffer (10 mM, pH 7.0) containing the 25-mer DNA strands (100 μM), 100 mM NaCl and 10% (v/v) methanol at 276 K with ν_{MW} = 9.44849(2) GHz.

form (α) was taken into account for the simulation. The simulations reproduced the experimental data as the parameters $\tau_c = 6.7 \times 10^{-11}$ s for only the radical (Fig. 3a) and 9.1×10^{-10} s for the DNA-bound radicals with $\alpha = 0.88$ (Fig. 3b) where the correlation time of DNA-free components is fixed as the former value. These results indicate that the tumbling of the radical moieties embedded in the duplex is slower than that of the unbound NCDNN. Under the concentration conditions, the fraction of 0.88 is in good agreement with that of 0.83 calculated from the association constant obtained from the CD titration. The line shape in the spectrum of the radical-DNA system approximated to that of only the radical by the displacement of NCDNN with NCD, indicating a competitive binding between NCD and NCDNN (Fig. S2†).

Encouraged by these findings, a radical-DNA complex has been expanded into a one-dimensional DNA system consisting of two 25-mer DNA strands 5′-(GCACTCGGA-CAGATTCTTGAGCTCT)-3′/5′-(TCTGTCGGAGTGCAG-AGCTCAAGAA)-3′, leading to the tandem repetitive array of double stranded DNA by the hybridization on sticky overhangs (Fig. 1c). Smear bands observed for the duplex on native PAGE analyses and the mobility shift with NCDNN showed the formation of one-dimensional DNA arrays holding the radical molecules (Fig. S3†). The intensity for the smear band of only the DNA array has the maximum at 200 bp in length with a dispersion (Fig. S3(b)†). As shown in Fig. 3c, the ESR spectrum of the NCDNN solution containing the 25-mer DNA strands exhibited an anisotropic feature more prominently than that of the radicals embedded in the 11-mer duplex, which is due to the size effect of the DNA array. These results have demonstrated the

self-assembly of the stable organic radicals on the one-dimensional DNA array.

To estimate the intermolecular spin–spin distance between the radicals embedded within the NCDNN/DNA complex (11-mer duplex) in a straightforward manner is of particular interest. Q-band four-pulse ELDOR (DEER) spectroscopy[10] applied to the complex gave two dominant distances at 1.8 and 2.0 nm (Fig. S4†). The latter was more dominating. Also, we have invoked molecular dynamics simulations for a model complex in which the radical sites are replaced by carbonyl groups,[10] acquiring the distributions of the distances (Fig. S5–S6†). Two dominating distances of 1.7 nm and 1.9 nm appeared and the latter is more prominent. The observed distances are in harmony with these calculated values, indicating that the radicals of the naphthyridine carbamate dimer are bound to the CGG/CGG triad as the addressable positions in the DNA duplexes.

The newly synthesized NCDNN is bound to the CGG/CGG triad in 10^5 affinity and delivered the two radicals on the DNA unit. The self-assembly of the DNA unit with sticky overhangs produced one-dimensional DNA arrays with multiple spins on. The DNA-spin unit would be useful for the construction of two- and three-dimensional DNA structures holding molecular spins at the programmed positions. The addressability of radical spins is of particular importance for spin–spin mutual microscopic arrangements in a controlled manner such as construction architecture for molecular spin-based quantum computers with a DNA backbone.[5,10]

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