Electronic Supplementary Material (ESI) for Chemical Communications

Ligand-induced electron spins-assembly on a DNA tile

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Supporting information

The contents of supporting information:

1) T_m measurement of two ssDNAs (D2/D1m) with NCD-TEMPO.

2) Circular dichroism (CD) titration of two ssDNAs (D2/D1m) with NCD-TEMPO.

3) ESR measurement of NCD-TEMPO in the presence of the DNA tile B with D1m strand.

4) Experimental section.

1) T_m measurement of two ssDNAs (D2/D1m) with NCD-TEMPO.

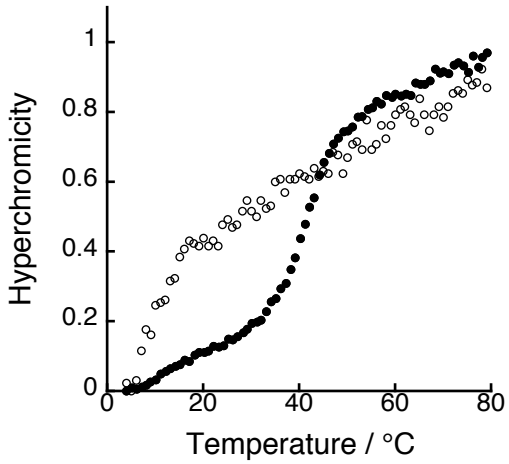

Fig. S1 Thermal denaturation profile of two ssDNAs (D2/D1m) (4.5 μM) 5'-d(TAA CGG AAA CGG AAT)-3'/5'-d(ATT CGG TAT CGG TTA)-3' in the (open circle) absence and (filled circle) presence of NCD-TEMPO (90 μM) in sodiumcacodylate buffer (10 mM, pH 7.0) containing sodium chloride (100 mM) and methanol (10% v/v).

2) Circular dichroism (CD) titration of two ssDNAs (D2/D1m) with NCD-TEMPO.

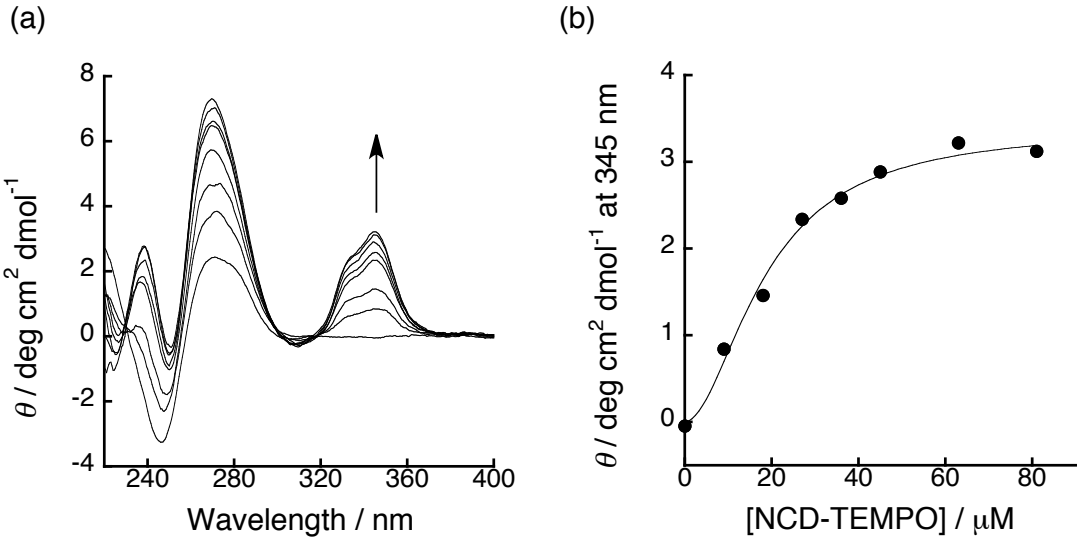

Fig. S2 (a) Circular dichroism spectra for the titration of two ssDNAs (D2/D1m) (4.5 μM) in sodium cacodylate buffer (10 mM, pH 7.0) containing sodium chloride (100 mM) and methanol (10% v/v) with the concentration of NCD-TEMPO at 0, 9.0, 18, 27, 36, 45, 63, and 81 μM. (b) Concentration dependence of the molar ellipticity at 345 nm. The closed circles and the solid line denote the observed values and the theoretical curve fitted to the Hill equation, respectively.

An apparent binding constant (K_{app}) of NCD-TEMPO with two single strands (D2/D1m) was calculated to be 1.2 x 10^4 M^{-1} by a titration experiment using circular dichroism (CD) (Fig. S2, ESI). However, it is difficult to quantitatively estimate a fraction of the ligand binding onto the DNA tile by the K_{app}, because there are some factors to define the fraction besides the ligand binding to DNA, for example, a fraction of induced-hybridization on the DNA tile by the ligand binding and DNA tile assembly.

3) ESR measurement of NCD-TEMPO in the presence of the DNA tile B with D1m strand.

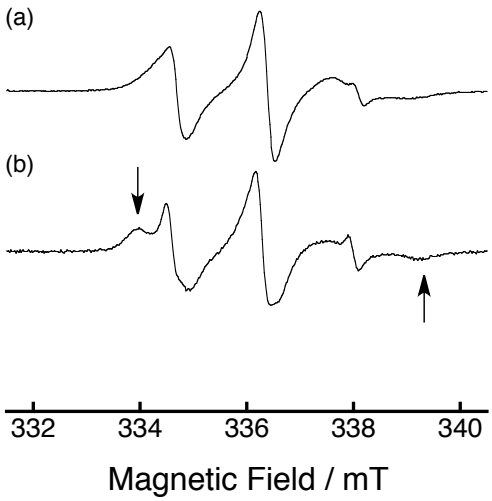

Fig. S3 The ESR spectrum of NCD-TEMPO (200 μM) in the presence of the (a) two ssDNAs (D2/D1m) (50 μM) and (b) DNA tile B unit (50 μM) with the D1m strand (50 μM) in TAE (1x) buffer containing magnesium acetate (12.5 mM), and methanol (10% v/v) at 295 K. Arrows indicate the slow motion component.

In our previous reports, the ESR spectrum of MBLs carrying an electron spin on one-dimensional DNA structure whose molecular size was approximately 200 base pairs did not significantly show the slow-motion component at 295 K.[1] The ESR spectrum of NCD-TEMPO on the tile B unit composing of 100 base pairs, however, showed the slow-motion component (Fig. S3b), indicating that the reason why the radical mobility on the DNA tile was much slower than that on the DNA duplex was likely due to the two-dimensional nature as well as the molecular size.

4) Experimental section.

Materials.

NCD-TEMPO was prepared according to previous report.[2] All single-stranded DNAs purified by HPLC were purchased from Life Technologies Japan Ltd..

Assembly of the DNA tiles.

The concentration of all oligonucleotides was adjusted to 100 μM by ultraviolet absorption at 260 nm in MilliQ on NanoVue (GE Healthcare). The strands of each tile unit were mixed and dissolved to 500 nM in Tris-acetate-EDTA (1 x TAE) buffer containing magnesium acetate (12.5 mM) (TAE/Mg^{2+}) where the final concentrations are given in the parentheses. The solution were annealed from 95 °C to 30 °C during 6 h in a TP 100 thermal cycler (TAKARA BIO INC.). Separately-prepared DNA tile A and B was mixed and annealed from 45 °C to 10 °C during 13 h.

Melting temperature (T_m) measurements.

NCD-TEMPO was dissolved in a sodium cacodylate (10 mM, pH 7.0) containing NaCl (100 mM), methanol (10% v/v) and D2/D1m (4.5 μM), where the final concentrations are given in the parentheses. The thermal denaturation profile was recorded on a UV-2550 spectrometer (Shimadzu) equipped with a TMSPC-8 temperature controller (Shimadzu). The absorbance of the sample was monitored at 260 nm from 4 °C to 80 °C with a sample heating rate of 1 °C/min.

Atomic force microscope (AFM) measurements.

20 μl DNA tile solution containing NCD-TEMPO were spotted onto freshly cleaved mica pretreated $NiCl_2$ (1 mM) and left to adsorb to the surface for 2 h. To remove excess salt, sample on the substrate was dropped. The DNA tile (250 nM) in TAE/Mg^{2+}, methanol (10% v/v), and Tween 20 (0.1% v/v) in the presence of D1f strand (2.5 μM), D1m strand (2.5 μM), and NCD-TEMPO (150 μM) with D1m strand (2.5 μM) was observed at room temperature. Imaging was done in a fluid cell with BL-AC40TS-C2 cantilever.

Electron spin resonance (ESR) measurements.

X-band solution CW-ESR measurements were performed with a BioSpin E500 (Bruker). Solution ESR spectra of NCD-TEMPO (200 μM) were measured in the presence of the two ssDNAs (D2/D1m) (50 μM) or DNA tile (50 μM) with D1m strand (50 μM) in TAE/Mg^{2+}, and methanol (10% v/v).

Sequence used in the assembly of DNA tile.

DNA tile was prepared according to procedure previously reported.[3] Single-stranded tail was shown in magenta.

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TAY1: GTAGCGCCGTTAGTGGATGTC
TAG2: GACTGCGTGTCAATGCTCACCGATCAACCAG
TAC2: TGTAGTATCGTGGCTGTGTAATCATAGCGGCACCAACTGGCA
TAR2: GATGGCGACATCCTGCCGCTATGATTACACAGCCTGAGCATTGACAC
TAB1: CTGACGCTGGTTGATCGGACGATACTACATGCCAGTTGGACTAACGG
TBG4: CGTCAGGCTGCTGTGGTCGTGC
TBY2: GCCATCCGTCGATACGGCACCATGATGCACG
TBB2: CGCTACCGTGCATCATGGACTAACCAGTGACCGCATCGGACAGCAGC
TBR4: GCAGTCGCACGACCTGGCGTTGTACTACGCAATCCTGCCGTATCGACG
TBD2: TATTTACAACGCCACCGATGCGGTCACTGGTTAGTGGATTGCGTAGTATAACGGAAACGGAAT
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References

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2 H. Atsumi, K. Maekawa, S. Nakazawa, D. Shiomi, K. Sato, M. Kitagawa, T. Takui, and K. Nakatani, *Chem. Lett.*, **2010**, *39*, 556–557.
3 E. Winfree, F. Liu, L. A. Wenzler and N. C. Seeman, *Nature*, **1998**, *394*, 539–544.