Programmed Assembly of Organic Radicals on DNA

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Electronic Supplementary Information

The contents of the supplementary information:

1) Synthesis of NCDNN.

2) T_m values of 11-mer duplexes in the absence and presence of NCDNN.

3) A Job's plot in CD spectra.

4) Simulation parameters for ESR spectra.

5) Displacement assay using NCD in ESR spectra.

6) Native PAGE analysis of a 1D DNA system.

7) Experimental evaluation of the spin-spin distances between the radicals embedded within the NCD-NN/DNA complex (11-mer duplex): Q-band four-pulse ELDOR (DEER) measurements.

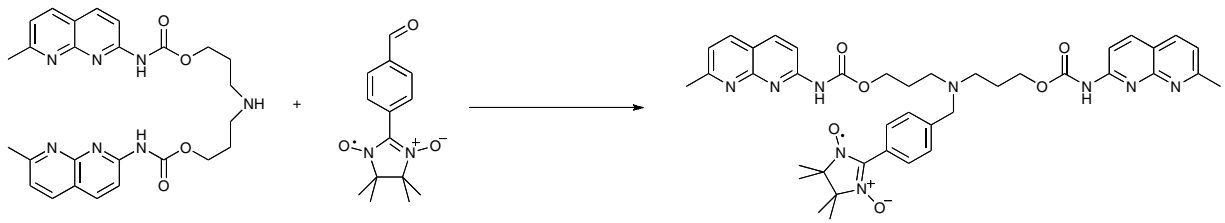
8) Distributions of the spin-spin distances as calculated by molecular dynamics simulation.

9) Atomic force microscopic experiments.

Supplementary Material (ESI) for Chemical Communications

1) Synthesis of NCDNN.

Scheme 1



Regents and conditions: $NaBH_3CN$, AcOH, MeOH, rt. 14 h.

NCD **1** and *p*-formylphenyl nitronyl nitroxide **2** were prepared according to procedures previously reported.[1,2] **1** (320 mg, 0.635 mmol) and **2** (330 mg, 1.27 mmol) were dissolved in methanol 25 ml, and acetic acid (330 μl) was added to the solution. After stirring at room temperature for 1.25 h, sodium cyanoborohydride (83 mg, 1.34 mmol) in methanol (15 ml) was added to the mixture. The solution was stirred at room temperature for 12 h. The resulting mixture was extracted with chloroform, and washed with aqueous $NaHCO_3$ and brine, and dried over $MgSO_4$. Purification by chromatography over silica gel (from 100:1 to 40:1 of chloroform/methanol) gave NCDNN (176 mg, 0.232 mmol, 35.6% yield) as a blue solid. HRMS (ESI): m/z calcd for $C_{40}H_{46}N_9O_6$ ($[M+Na]^+$) 771.3470, found 771.3468. Elemental Anal. Found: C, 58.28; H, 5.86; N, 14.99. Calc. for $C_{40}H_{46}N_9O_6 \bullet 0.75CH_3Cl$: C, 58.38; H, 5.62; N, 15.04%.

The NMR spectra of radical molecules in a solution state can not be clearly observed due to paramagnetic relaxations for a conventional NMR spectroscopic method to characterize closed-shell molecules in organic syntheses, and therefore the characterization of NCDNN has been conducted on the mass spectrometry and the elemental analysis.

3) T_m values of 11-mer duplexes in the absence and presence of NCDNN.

Table S1. T_m values of the duplexes.[a]

X-Y	T_m(-) / ℃	T_m(+) / ℃	ΔT_m / ℃
A-A	19.5	18.2	-1.3
C-C	20.0	21.2	1.1
G-G	24.8	38.3	13.5
T-T	26.7	28.4	1.7
A-C	17.6	17.2	-0.4
C-T	23.2	23.1	0.0
G-A	26.9	32.2	5.2
G-T	28.4	29.6	1.1
G-C	39.6	40.2	0.5
A-T	34.3	35.4	1.1

[a]The Melting curves were measured for the 11-mer duplex (4.5 μM) of 5'-(CTAA**CXG**AATG)-3'/5'-(CATT**CYG**TTAG)-3' in sodium cacodylate buffer (10 mM, pH 7.0) containing 100 mM NaCl and 10% (v/v) methanol. T_m(-) and T_m(+) represent melting temperature in the absence and presence of NCDNN (45 μM), respectively. ΔT_m is calculated as T_m(+) - T_m(-).

3) A Job's plot in CD spectra of NCDNN and DNA system.

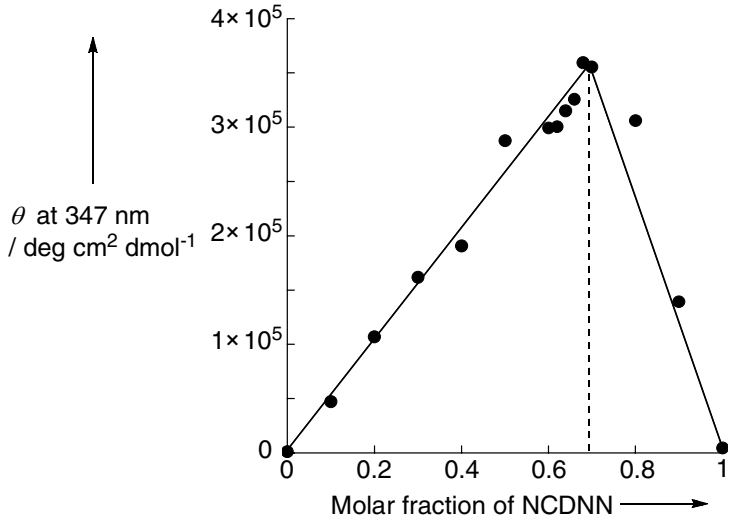


Figure S1. A Job's plot obtained by monitoring the molar ellipticity at 347 nm, plotted against the molar fraction of NCDNN. Closed circles and a solid line denote the observed values and an eye guide, respectively. Total concentration of NCDNN and a duplex was 50 μM in sodium cacodylate buffer (10 mM, pH 7.0) containing 100 mM NaCl and 10% (v/v) methanol. The crossover point was 0.67, which suggests that the stoichiometry is in the ratio of NCDNN: DNA = 2: 1.

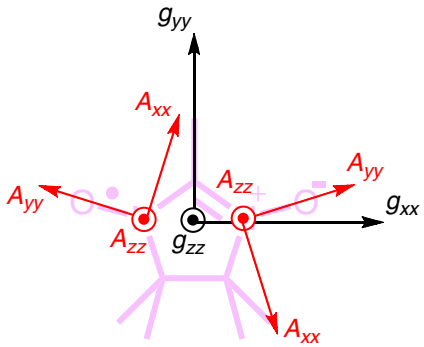
4) Simulation parameters for ESR spectra.

The spectral simulation was performed using a program package, *Easy Spin* (*Ver*. 2.7.1),[3] working on the MATLAB software.

Table S2 Simulation parameters for ESR spectra of only NCDNN and a NCDNN-DNA complex.

	NCDNN	[a]NCDNN-DNA
g_{xx}	2.0098	2.0098
g_{yy}	2.0053	2.0061
g_{zz}	2.0042	2.0032
[b]g_{ave}	2.0064	2.0064
A_{xx} / mT	0.04951	[d]0.04951
A_{yy} / mT	0.18435	[d]0.18435
A_{zz} / mT	2.19725	[d]2.19725
[c]A_{ave} / mT	0.81037	0.81037
ΔB_{FWMH} / mT	0.11494	0.09253
τ_c / s	6.6545×10^{-11}	9.1332×10^{-10}
α		0.88

[a]The parameters for the hyperfine couplings, the line width and the rotational correlation time on the DNA-free components were fixed as that of only NCDNN. [b]$g_{ave} = (g_{xx}+g_{yy}+g_{zz})/3$. [c]$A_{ave} = (A_{xx}+A_{yy}+A_{zz})/3$. The two nitrogen nuclei on the nitronyl nitroxide moiety were assumed to be equivalent. [d]The principal values of the hyperfine couplings were set as the parameter obtained from the simulation of only NCDNN. The directions of the principal axes for hyperfine and *g*-tensors were fixed as depicted below.



5) Displacement assay using NCD in ESR spectra.

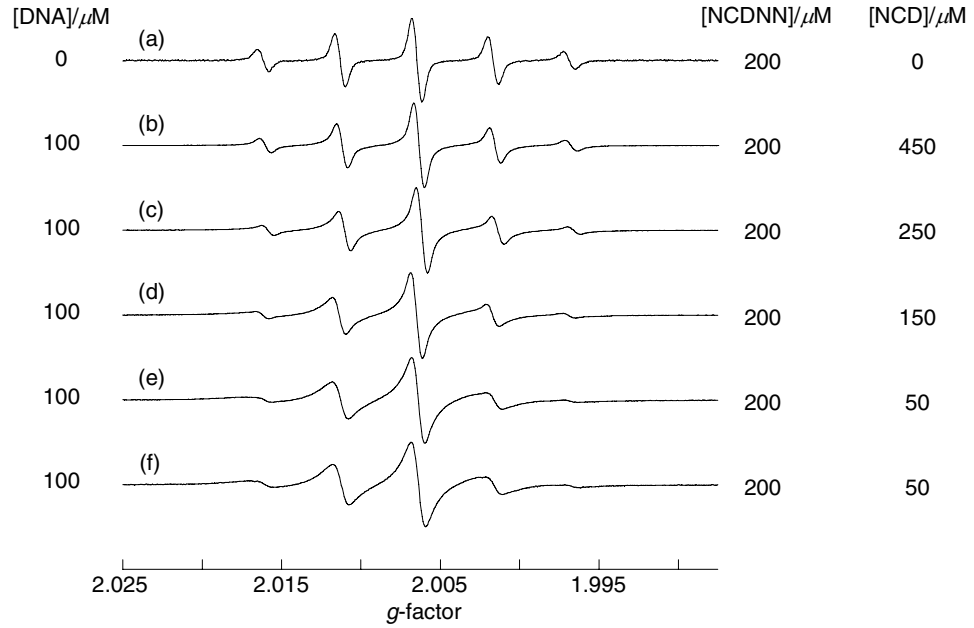


Figure S2. ESR spectra of NCDNN (200 μM) in the (a) absence and (f) presence of the DNA duplex (100 μM). Panels (b), (c), (d) and (e) show the displacement assay of NCDNN (200 μM) with NCD (450, 250, 150, 50 μM) in the duplex (100 μM). The spectra were recorded in sodium cacodylate buffer (10 mM, pH 7.0) containing 100 mM NaCl and 10% (v/v) methanol at 297 K.

6) Native PAGE analysis of a 1D DNA system.

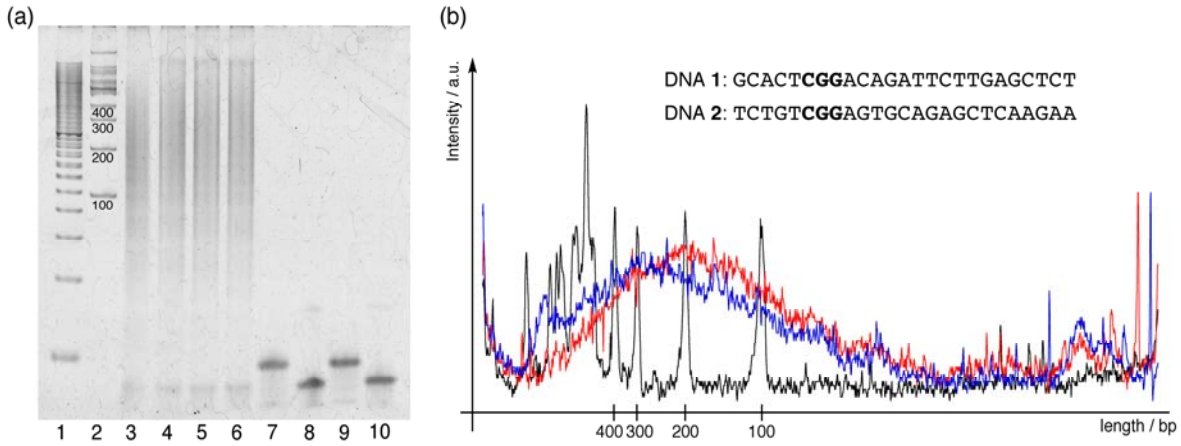


Figure S3. Native PAGE of the 25-mer DNA strands 5'-(GCACT**CGG**ACAGATTCTTGAGCTCT)-3'/5'-(TCTGT**CGG**AGTGCAGAGCTCAAGAA)-3' in a buffer containing 100 mM NaCl and 10 mM sodium cacodylate (pH 7.0) at 3 °C. (a) Lanes 1 and 2, 20 and 100 base pair DNA ladders as size markers, respectively; Lanes 3-6, DNA **1**·**2** (4.5 μM) with NCDNN (0, 9, 18, 45 μM); Lanes 7 and 8: DNA **1** and **2** (4.5 μM); Lanes 9 and 10, DNA **1** and **2** (4.5 μM) with NCDNN (45 μM). (b) Black, red and blue lines represent band intensity for lane2, 3 and 6. The maximum of the band intensity for lane 3 has been found at 200 bp in length, and the dispersion is estimated as 370 bp in width between the half maxima.

7) Experimental evaluation of the spin-spin distances between the radicals embedded within the NCDNN/DNA complex (11-mer duplex): Q-band four-pulse ELDOR (DEER) measurements.

High-power four-pulse ELDOR (DEER) spectroscopy was applied to evaluate the spin-spin distances between the radicals embedded within the NCDNN/DNA and those from unlaced radical pairs. The ELDOR experiments were carried out at Q-band on a Bruker ELEXSYS E580 pulsed ESR spectrometer equipped with a large-capacity helium cryostat (made-to-order Optibath SXM, Oxford Instruments, INC.) and a temperature controller system (ITC 503, Oxford Instruments, INC.).

Frozen-solution CW and pulsed ESR/ELDOR spectra of NCDNN (1 mM) in the presence of the DNA duplex (2 mM) were recorded in sodium cacodylate buffer (20 mM, pH 7.0) containing 200 mM NaCl and 10% (v/v) methanol at 50 K.

All pulses were amplified via a high-power pulsed traveling wave tube (TWT) amplifier (made-to-order, Applied Systems Engineering, INC). Four-pulse ELDOR experiments were performed with the pulse sequence of $\pi/2(\nu_A)$-τ_1-$\pi(\nu_A)$-(τ_1+t)-$\pi(\nu_B)$-(τ_2-t)-$\pi(\nu_A)$-τ_2-echo. The detection pulse of ν_A was set at 16 ns for the $\pi/2$ pulse at the center of the resonator dip. The pump pulse of ν_B was set to 62 ns and applied at 80 MHz-higher frequency which corresponds to the intensity maximum of the field-swept echo-detected ESR spectrum from the nitroxide complex, as indicated in Fig. S4(a). τ_1 and τ_2 were set 200 and 500 ns, respectively. All the spectra were recorded in the frozen solution of toluene at 50 K with an experiment repetition time of 1 ms. 32000 scans were accumulated with 269 data points and time increments Δt of 2 ns giving an approximate measurement time of 2.4 h. Experimental data were analyzed with DeerAnalysis2006.[4] The data were background corrected using a third order polynomial background function in DeerAnalysis2006. The distance distributions were obtained by Tikhonov regularization in the range of 1.6 and 8.0 nm using a regularization parameter of 1. Two dominant distances were obtained at 1.75 and 2.03 nm. The other three small peaks were observed at 2.41, 3.04, and 3.64 nm, as shown in Fig. S4(c). The two dominant distances correspond to those attributed to two pairs of the radicals within the complex: The experimental result is in harmony with the distances expected from the molecular dynamics simulation described below in 8 (see Fig. S5). The other minor three distances likely arise from pairs of the radicals that are not constrained within the complex such as an intermolecular pair of the "free" radicals. Thorough and detailed pump and probe survey of the DEER experiments are underway to get insights into relative orientations between the radicals constrained within the complex.

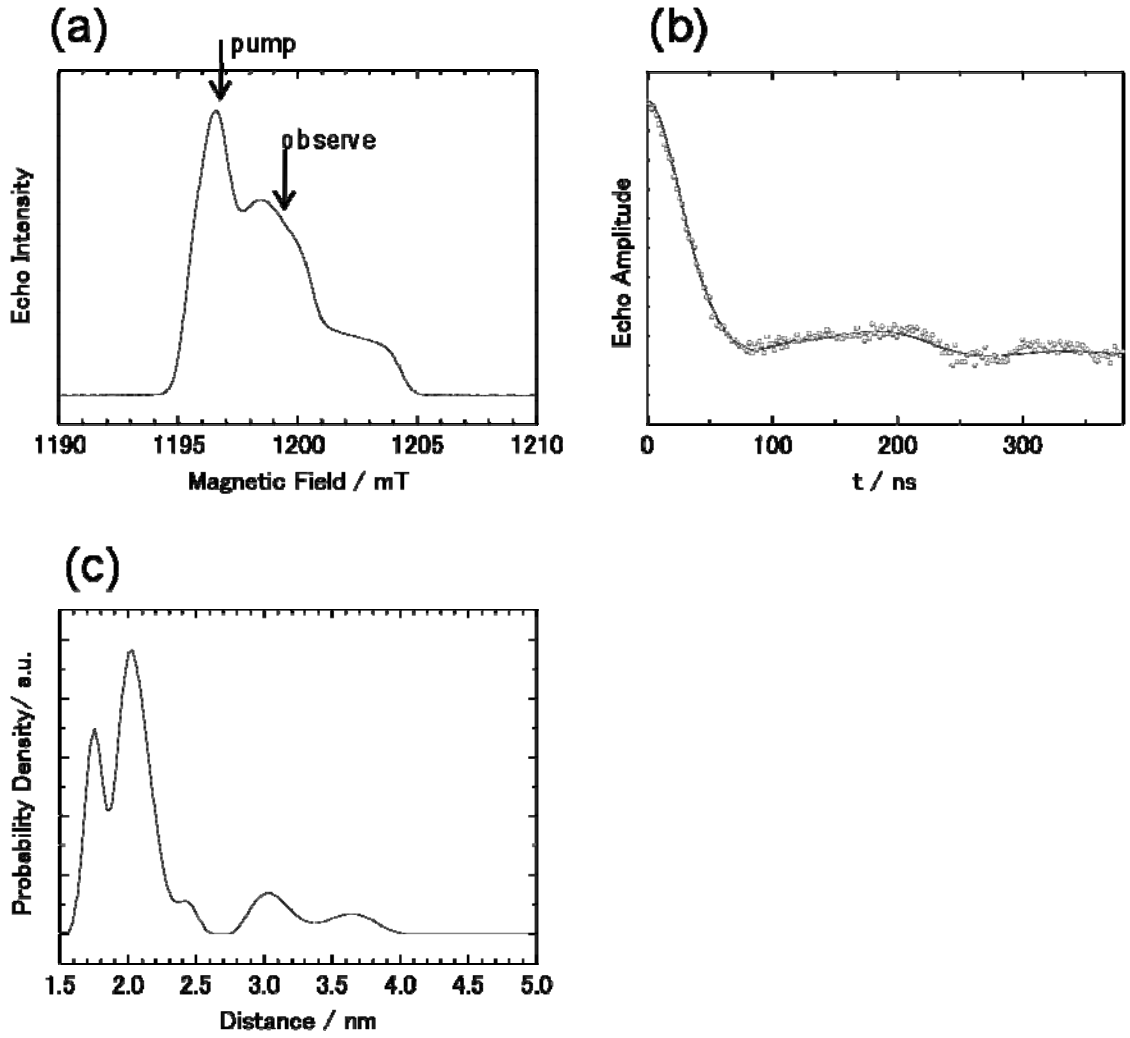


Figure S4. Experimental determination of the spin-spin distances between the radical sites within the NCDNN/DNA complex (11-mer duplex) by Q-band four-pulse ELDOR (DEER) spectroscopy in the sodium cacodylate buffer (20 mM, pH 7.0) containing 200 mM NaCl and 10% (v/v) methanol at 50 K. (a) Echo-detected field-swept ESR spectrum with marked "pump" and "observe," i.e., pump and probe positions, respectively in the DEER experiments. Microwave frequency: 33.63542 GHz. (b) Time-domain DEER data. The background curve was subtracted in terms of 3rd order polynomial function. (c) Distance distribution obtained by Tikhonov regularization.

8) Distributions of the spin-spin distances as calculated by molecular dynamics simulation.

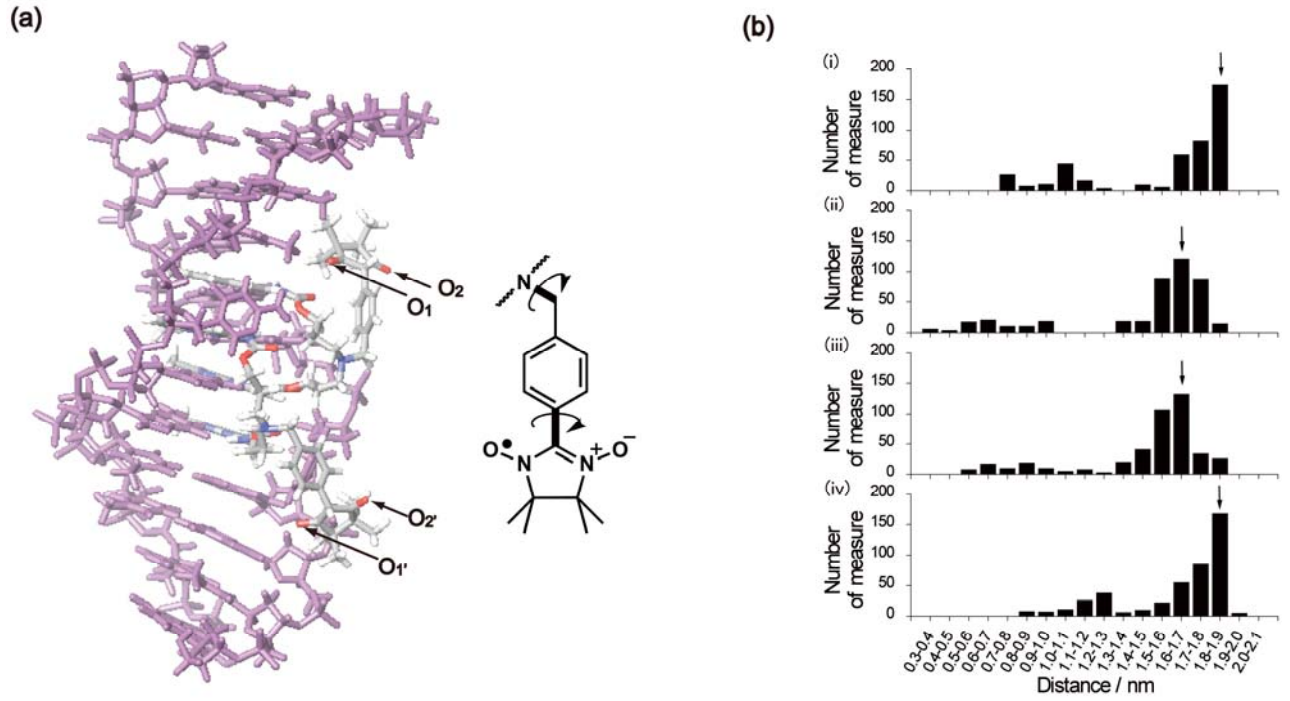


Figure S5. (a) A structure of the NCDNN/DNA complex which generates 44 initial structures for the molecular dynamics simulation (left). Bold lines indicates the bonds rotated for generating the 44 initial structures (right) (b) Distributions of the distances between carbonyl oxygen atoms (i) O_1-$O_{1'}$, (ii) O_1-$O_{2'}$, (iii) O_2-$O_{2'}$, and (iv) O_2-$O_{1'}$.

Figure S5(a) shows a structure of NCDNN/DNA complex for the molecular dynamics simulation. For the simulation, two CO groups were substituted for the N-O groups. Following the method below, we have carried out molecular dynamics simulation at 300 K for 10 ps from the 44 initial structures, obtaining totally 440 structures. The distances between carbonyl oxygen atoms were measured from the structures. The distributions of the distances are shown in Figure S5(b).

Method for molecular dynamics simulation

1. Two C-O groups were substituted for two N-O groups in NCDNN.

1. Each of four bonds in the NCD-NN/DNA complex was rotated by a 30 degree increment to produce initial 44 structures (11 structures × 4 bonds). Energy minimization of the 44 structures was carried out with MacroModel (version 7.5) using Amber* force field. The terminal base pairs were kept hydrogen bonded by constraining the distance.

3. Molecular dynamics simulation was done at 300 K for 10 ps. During the simulation, 10 structures were monitored every 1 ps and were saved. Total 440 structures were obtained for the measurement of distances between two oxygen atoms. The distances between carbonyl oxygen atoms (O_1-$O_{1'}$, O_1-$O_{2'}$, O_2-$O_{2'}$, and O_2-$O_{1'}$) were measured, and the distributions of the distances were analyzed (Figure S5(b)).

9) Atomic force microscopic experiments.

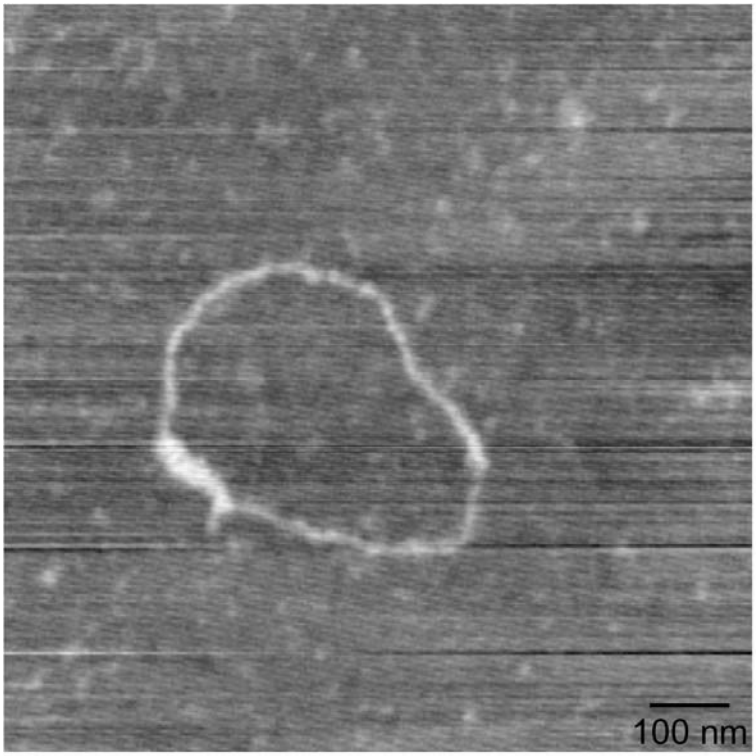


Figure S6. AFM images showing pRSET/EmGFP based vectors in air.

Atomic force microscopic experiments were conducted to investigate the size of aggregates for the 25-mer DNA strands, which is expected to be a 200 bp length at the maximum distribution of the aggregates from the gel analyses. In figure S6 is displayed the AFM image of the plasmid vectors as a positive control, evidently indicating a circular feature, however the examination for the 25-mer DNA strands could not make the aggregates of it clear (data not shown).

References

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