Supporting information

CW-EPR measurements

CW-EPR spectra were recorded of the \( \chi \) spin label and the noncovalently spin labeled dsDNA 1 in phosphate buffer with 20% (v/v) ethylene glycol (Figure S1). As the temperature of the \( \chi \) spin label sample is decreased the linewidth becomes broader due to the decreased rotational correlation time of the spin labels. At -40 °C the sample is completely frozen and the spectrum resembles a rigid nitroxide spectrum as can be seen from the spectrum recorded at -173 °C (Figure S1). As the temperature for the sample of noncovalently spin labeled DNA is decreased from 0 to -10 °C peaks that belong to transitions from spin labels with slow tumbling rate become clear as shown by the * in Figure S1. Since these peaks are not visible in the \( \chi \) spin label sample at -10 °C they must belong to spin labels that are bound to DNA, which has a slower tumbling rate in solution. As the temperature is decreased further the intensity of the peaks from slow tumbling spin labels increases, indicating that the fraction of bound spin labels increases with decreased temperature. At -30 °C no contribution from fast tumbling/unbound spin labels is observed, as indicated by the reduced intensity of the corresponding transitions (Figure S1, +). It was observed that both the DNA and the \( \chi \) spin label samples start to freeze at ~ -30 °C and become completely frozen at -40 °C. It's possible that the freezing of the sample at -30 °C has an impeding effect on the binding of spin labels to the DNA abasic sites.
Figure S1. CW-EPR spectra of \( \varsigma \) spin label (left) and noncovalently spin labeled dsDNA 1 (right) recorded at 0 to -40 °C. The spectrum in dashed line is from the free \( \varsigma \) spin label at -173 °C. Samples were dissolved in phosphate buffer with 20% ethylene glycol. * and + symbols show EPR transitions from spin labels bound and unbound to DNA, respectively.

Simulation of PELDOR time traces

PELDOR time traces and distance distributions were simulated using a geometrical vector model. The conformational distribution and dynamics of the spin labels was modeled using the cooperative twist-stretch dynamic model for short dsDNA (14). Table S1 summarizes the distribution in geometrical parameters used to simulate the PELDOR time traces. The spin labels and their equilibrium positions were represented by vectors, positioned relative to the center of the DNA helix axis. The equilibrium position of the spin labels was obtained from a molecular model of the DNA duplexes with \( \varsigma \) spin labels docked into the abasic sites (Figure S2). The position of the spin labels was parameterized by defining three variables: DNA radius (R), DNA length (L) and torsion...
angle (φ). The equilibrium value for R was defined as the distance the spin labels are shifted away from the center of the DNA helix axis (Figure S2b). R was allowed to have a standard deviation of 0.65 Å as previously determined (1). The equilibrium value for the DNA length was defined as the height between spin labels (Figure S2b) and the distribution in L was given by Eq. S1 (1) where $\sigma_R$ is the standard deviation in R and n is the number of base pairs between the spin labels.

$$\sigma_L = \frac{\sigma_R \cdot n}{-3.2}$$  \quad \text{Eq. S1}

The equilibrium angle between the N-O axes of the spin labels φ was determined from the DNA molecular models. The distribution (two standard deviations) in φ was previously determined to 22° per helical turn (1). Since the spin labels in dsDNA 1 and dsDNA 2 are approximately 1 and 2 helical turns apart, respectively, the distribution in φ for dsDNA 1 and dsDNA 2 was determined to be 22° and 48°, respectively. The length of the vectors representing the spin labels $L_\varsigma$, defined as the distance between the center of the N-O bond and the other end of the spin label (Figure S2b), was 11 Å. The equilibrium values for the height and torsion angle between spin labels were adjusted slightly until a good fit to the modulation frequency was obtained. The equilibrium values and distributions in R, L and φ for dsDNA 1, dsDNA 2 and dsDNA 2 +LacI are summarised in Table S1.

![Figure S2. Molecular model of DNA and definition of geometric parameters. a) Molecular model of dsDNA 1 with φ spin labels docked inside abasic sites. The spin labels are dark grey. The height between the spin labels, assigned as the DNA length parameter is shown. b) φ spin label with illustrations defining the DNA radius R and spin label length $L_\varsigma$.](image-url)
Table S1. Geometric parameters used in the molecular model for the simulation of the PELDOR time traces.

| dsDNA         | DNA Radius (R) [Å] | DNA Length (L) [Å] | Torsion (φ) [˚] |
|---------------|--------------------|--------------------|------------------|
| dsDNA 1       | 2.7 ± 0.65(0.01)   | 37.2 ± 2.23(0.03)  | 75 ± 11(0.2)     |
| dsDNA 2       | 2.7 ± 0.65(0.01)   | 70 ± 4.51(0.03)    | 60 ± 24(0.2)     |
| dsDNA 2 + LacI| 2.7 ± 0.65(0.01)   | 65.4 ± 5.20(0.03)  | 75 ± 24(0.2)     |
| dsDNA 3       | 2.7 ± 0.65(0.01)   | 67.6 ± 4.51(0.03)  | 60 ± 24(0.2)     |
| dsDNA 3 + LacI| 2.7 ± 0.65(0.01)   | 62.1 ± 5.05(0.03)  | 80 ± 24(0.2)     |

*The DNA radius, DNA length and torsion angle (angle between the spin labels N-O axis) are given as mean value ± one standard deviation. The uncertainty in the standard deviation is given within brackets.*
LacI-induced bending of DNA covalently spin labeled with Ç spin labels

To evaluate the effects from abasic sites and non-covalent spin labeling on the PELDOR measurements of dsDNA 2 the same 29-mer DNA sequence was covalently spin labeled with the spin label Ç (Table S2) (2) and measured with PELDOR at 40-90 MHz frequency offsets, both before and after addition of LacI.

Table S2. Sequence for the dsDNA covalently spin labeled with Ç.

| DNA sequence       |
|--------------------|
| dsDNA 3 5'-GCGÇATTTGAGCGGATAACATTGGCG-3' |
| 3'-CGCGTAAACACGCCCTATTGTTAAACCAG-5' |

The spin labels are denoted by Ç. The bold sequence in dsDNA 3 is the 19-mer Lac repressor consensus sequence.

The PELDOR time traces of dsDNA 3 with and without LacI show a pronounced modulation at almost all frequency offset and a modulation depth of about 50% at 40 MHz offset (Figure S3a, b). The Fourier transformed time traces show an increased parallel component as the frequency offset is increased from 40 to 90 MHz, which is in agreement with dsDNA 2 (Figure S3c, d). Analyzing the orientation averaged time traces with Tikhonov regularization yields a mean interspin distance of 68.1 and 62.1 Å for dsDNA 3 and dsDNA + LacI, respectively (Table S3). The change in interspin distance is clearly seen when comparing the orientation averaged time traces and corresponding distance distributions for dsDNA 3 and dsDNA 3 + LacI (Figure S4). Using trigonometry and these mean distances the bending angle of the dsDNA 3 bound to LacI can be estimated as 48.5°. This is 6.5° larger bending than for the non-covalently spin labeled DNA, dsDNA 2.

The PELDOR time traces for dsDNA 3 were also simulated using the same dynamics model as for simulations of time traces for dsDNA 2 and the bending angle determined from the orientation averaged time traces of dsDNA 3 (Table S1). The simulated time traces have an excellent fit to the experimental time traces and the distance distributions from the simulation model fits nicely to the distance distribution from the orientation averaged PELDOR time trace (Figure S3e, f and Table S3). PELDOR measurements on dsDNA 3 show the mean interspin distance to be 1.1 and 2.5 Å shorter than for the non-covalently spin labeled DNA, dsDNA 2. The small difference in the mean interspin distance and LacI induced bending between covalently and non-covalently spin labeled DNAs is most likely due to the structural perturbation from the abasic sites and the exact position of the spin labels within the DNA duplex.
Figure S3. PELDOR data for covalently spin-labeled dsDNA 3 with and without LacI at 40 to 90 MHz offsets. a,b) Background corrected PELDOR time traces of dsDNA 3 without and with LacI (black), respectively, overlaid with the corresponding simulated time traces (red). The time traces have been displaced on the y-axis for clarity. Original time traces are shown in figure S5. c) and d) are the respective Fourier transformations of the time traces in a) and b). The black spectra are the Fourier transformations of the respective summed time traces shown in e) and f). e) and f) are the summed PELDOR time trace of dsDNA 3 without and with LacI, respectively. The inset shows the distance distribution obtained from the summed time traces using DeerAnalysis (blue) and the distribution obtained from the model based simulations.
Figure S4. Overlay of the PELDOR data for dsDNA 3 and dsDNA 3 + LacI. a) Background corrected orientation averaged time traces. b) Distance distributions from the time traces in a) obtained by Tikhonov regularization.

Table S3. Interspin distances for dsDNA 3

| dsDNA      | $r_{DA}$ [Å]$^a$ | $r_{Simulation}$ [Å]$^b$ | $r_{MM/X-ray}$ [Å]$^c$ |
|------------|------------------|--------------------------|------------------------|
| dsDNA 3    | 68.1, 4.0        | 68.0, 4.8 (1, 0.1)       | 73                     |
| dsDNA 3 + LacI | 62.1, 4.1      | 62.2, 5.3 (1, 0.1)       | 67                     |

$^a$ Most probable distance, standard deviation from orientation averaged PELDOR time traces using DeerAnalysis 2011. $^b$ Most probable distance, standard deviation. The error in the distance, standard deviation is in brackets. $^c$ Interspin distances obtained from molecular modeling using B-form DNA duplexes. The interspin distance for dsDNA 3 bound to LacI was estimated by modeling spin labels into the X-ray structure of a 21-mer dsDNA bound to LacI (pdb id. 1LBG).
Original PELDOR time traces

**Figure S5.** Original PELDOR time traces of noncovalently spin labeled a) dsDNA 1, b) dsDNA 2, c) dsDNA 2 + LacI, d) Ç spin labeled dsDNA 3 and e) Ç spin labeled dsDNA 3 + LacI.
References

1. Marko, A., Denysenkov, V., Margraf, D., Cekan, P., Schiemann, O., Sigurdsson, S.T. and Prisner, T.F. (2011) Conformational Flexibility of DNA. *J. Am. Chem. Soc*, **133**, 13375–13379.

2. Schiemann, O., Cekan, P., Margraf, D., Prisner, T.F. and Sigurdsson, S.T. (2009) Relative Orientation of Rigid Nitroxides by PELDOR: Beyond Distance Measurements in Nucleic Acids. *Angew. Chem. Int. Ed*, **48**, 3292–3295.