

# **Structure of p53 binding to the BAX response element reveals DNA unwinding and compression to accommodate base-pair insertion**

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## SUPPLEMENTARY DATA

### SUPPLEMENTARY METHODS

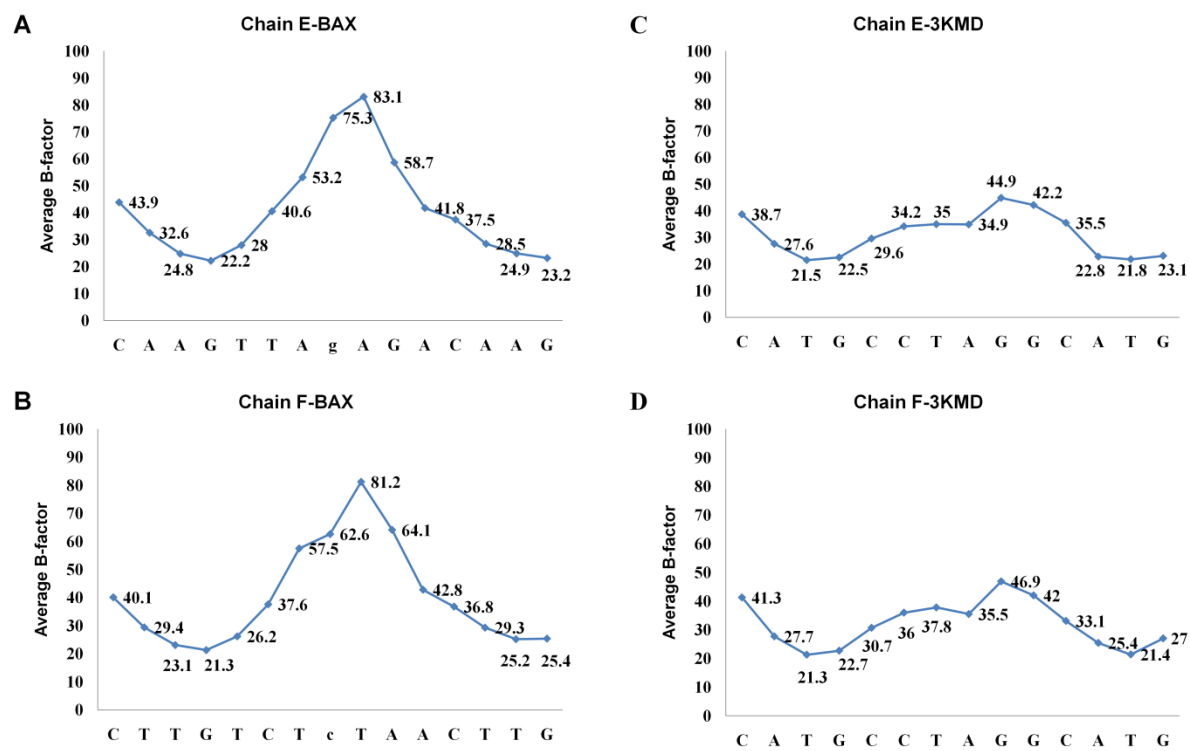
#### *EPR measurement of solution conformation of the bound DNA*

*DNA spin labeling:* Nitroxide spin labels were attached to specific sites at BAX-RE using a nucleotide-independent phosphorothioate scheme (1,2), and labeling sites used in this study are shown in Supplementary Figure S5. DNA oligonucleotides containing phosphorothioate modifications at the desired nucleotide(s) were synthesized by solid-phase chemical synthesis (IDT, Coralville, IA). The modified DNA strand was subsequently coupled with a reactive nitroxide precursor, resulting in covalent attachment of a probe designated as R5 (1-oxyl-2,2,5,5-tetramethylpyrroline). Labeled DNA oligonucleotides were purified using anion-exchange HPLC, which eliminates failed oligonucleotide fragments from synthesis, removes excess spin label and the small amount of unlabeled full-length DNA. HPLC fractions containing purified labeled DNA were desalted using a C18 reverse-phase column (Alltech Biotechnology, Inc. Deerfield, IL), lyophilized, then suspended in water and stored at  $-20^{\circ}\text{C}$ . Final concentrations of labeled DNA were determined by UV absorption at 260 nm using extinction coefficients of  $211,400\text{ M}^{-1}\text{cm}^{-1}$  for chain E and  $197,700\text{ M}^{-1}\text{cm}^{-1}$  for chain F. Note that following previously validated protocols (1,2), studies reported here were carried out without separating the  $R_p$  and  $S_p$  phosphorothioate diastereomers present at each attachment site.

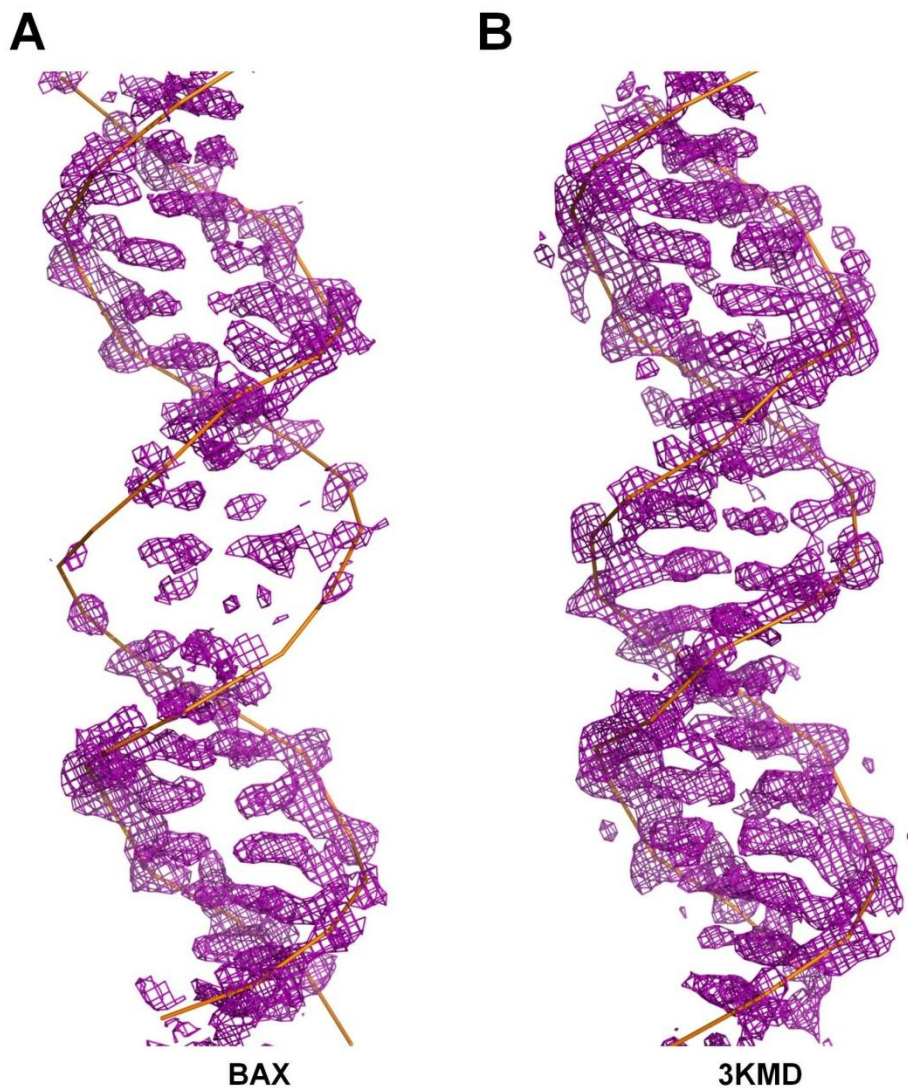
*Preparation of EPR sample:* DNA duplexes each containing one pair of R5's, were prepared by annealing appropriate oligonucleotide strands in a 1:1 ratio unless otherwise stated. For annealing, DNAs were first heated at  $95^{\circ}\text{C}$  for 1 minute then cooled at room temperature for 20 seconds. Proper amount of salts were then added to obtain a solution with 50 mM HEPES (2-[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid, pH 7.5), 100 mM NaCl, 5 mM  $\text{MgCl}_2$ , with the labeled DNA duplex being approximately 200  $\mu\text{M}$ . This mixture was let standing at room temperature overnight. To prepare the p53/BAX-RE complex, a labeled DNA duplex from the stock solution mentioned above was mixed with the p53 core domain in a 1:5 ratio in a solution containing 50 mM HEPES (pH 7.5), 100 mM NaCl, 5 mM  $\text{MgCl}_2$ , 10  $\mu\text{M}$  ZnOAc, and 40% (v/v) glycerol. After incubating at  $4^{\circ}\text{C}$  for 1 hour, the final sample, which contains approximately 50  $\mu\text{M}$  of tetrameric protein-DNA complex, was used for EPR measurements immediately (see below).

*Pulsed EPR spectroscopy for measuring inter-nitroxide distances:* Inter-nitroxide distance was measured using Double Electron-Electron Resonance (DEER) spectroscopy (3). For each DEER measurement, 25  $\mu\text{l}$  of each sample prepared as described above was placed in a round quartz capillary (2.0 mm ID, 2.4 mm OD, Vitrocom, Inc., Mountain Lakes, NJ) sealed at one end, and was then flash-frozen in liquid nitrogen. Measurements were carried out at 80 K on a Bruker ELEXSYS E580 X-band spectrometer equipped with an ER4118-MD4-EN resonator. A dead-time free four-pulse scheme was used (3), with the pump pulse frequency set at the center of the nitroxide spectrum and the observer frequency being approximately 70 MHz higher. The observer  $\pi$  pulse was 32 ns. The pump  $\pi$  pulse was optimized using a nutation experiment (4) and was usually set at 32 ns. The video bandwidth was fixed at 20 MHz. The shot repetition time was set at 1000  $\mu\text{s}$  based on measured  $T_1$  of approximately 600-800  $\mu\text{s}$  (5). Accumulation time in each measurement ranged from 8-24 hours with 100 shots per point. Inter-spin distance distributions were computed from the resulting dipolar evolution data using DEERanalysis2011 (6). The average distance ( $r_0$ ) and the standard deviation of the distribution ( $\sigma$ ) were computed as previously described (2). Based on repeated measurements, errors in measured  $r_0$  were less than 1  $\text{\AA}$ . Note that  $r_0$  errors report on uncertainty in the measured average distances, and they are distinct from the  $\sigma$  values that characterize the width of inter-spin distance distribution. Control experiments with singly-labeled DNA duplexes gave no measurable spin dipolar interaction, confirming the DEER data indeed report desired intra-molecular distances, and are not biased by artifacts due to inter-molecular spin-spin interactions.

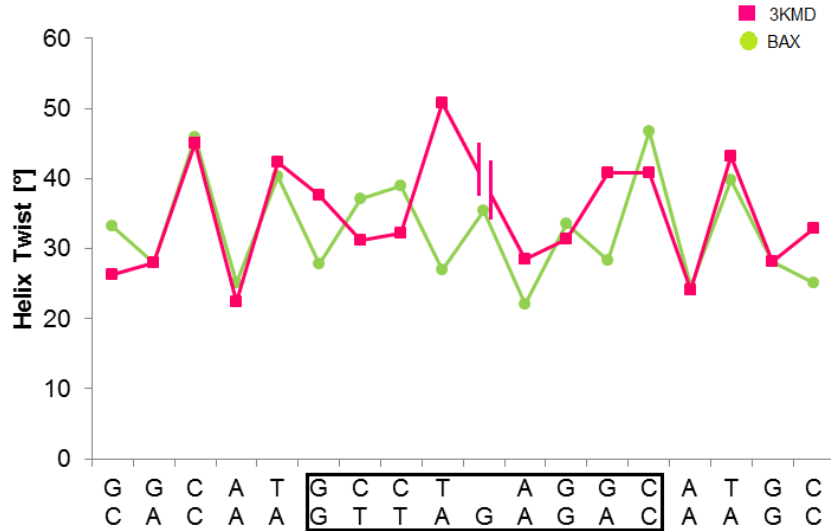
*Computation of expected inter-R5 distances:* The previously validated NASNOX program (1) was used to compute expected inter-R5 distances at desired sites within the BAX complex crystal structure, with a slight modification implemented in the program to account for the presence of protein atoms. For each distance set, allowable R5 conformers present as  $R_p$  or  $S_p$  diastereomers (i.e., attached to the O1P or O2P atom) at the corresponding nucleotides were identified using the following search parameters: t1 steps: 3; t2 steps: 6; t3 steps: 6; fine search: on; t1 starting values:  $180^\circ$ ; t2 starting values:  $180^\circ$ ; t3 starting values:  $180^\circ$ ; and no additional conformer search criterion (i.e., hydrophobic contacts) (see (1) for details on these parameters). Using the two ensembles of the allowable conformers identified, inter-R5 distance was computed between the nitrogen atoms at each nitroxide. Controls showed that varying the t1, t2, and t3 parameters resulted in less than 1  $\text{\AA}$  difference in the predicted averaged inter-R5 distances.



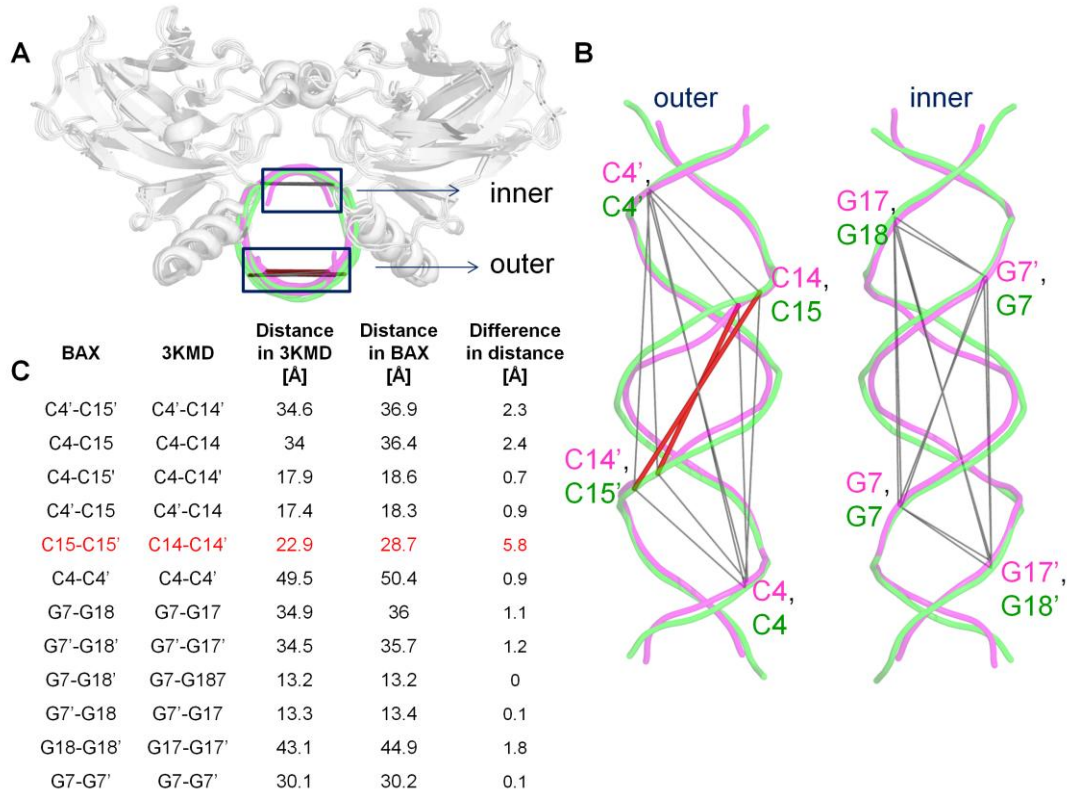
**Supplementary Figure S1: B-factor distribution of DNA in BAX (A, B) and 3KMD (C, D) structures.** The high B-factor in the central region of BAX DNA indicates that this region is disordered.



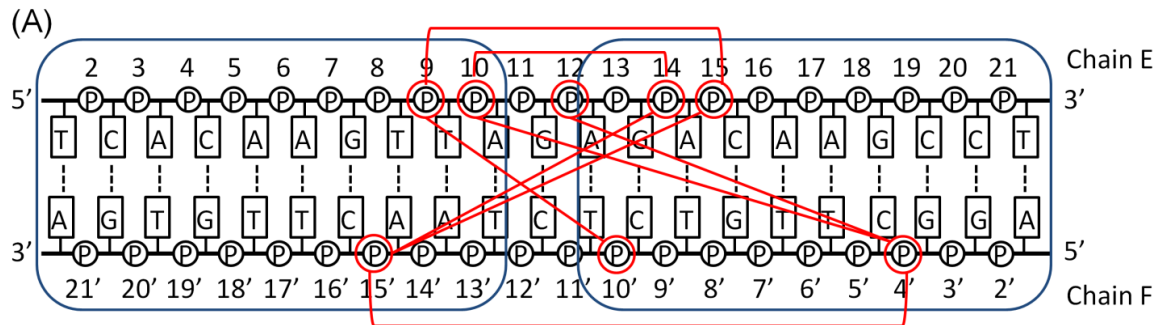
**Supplementary Figure S2: *Fo-Fc* omit map of DNA in the p53/BAX-RE complex (A) and 3KMD structure (B).** The electron density is contoured at  $2\sigma$  and is superimposed with the final model.



**Supplementary Figure S3:** DNA structural parameters for 3KMD and BAX DNAs. Plots compare helix twist values between base pairs of the 3KMD (magenta) and BAX (green) DNA structures. It is noteworthy that the average helix twist between the inner C/G base pairs of the CWWG core elements (frame), is 31.3° for BAX compared to 36.1° for 3KMD, indicating the untwisting of the BAX DNA upon p53 binding.

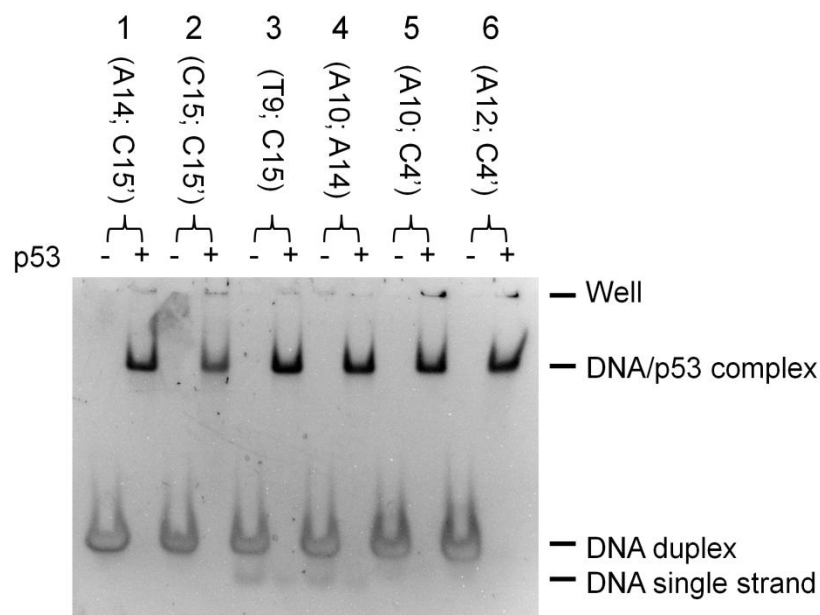


**Supplementary Figure S4:** (A) The p53/DNA interface is located on one side the double helix. The BAX RE forms an interface with the p53 core domain tetramer that is almost identical to a binding site without spacer. This is possible because the required conformational adjustment of the DNA occurs at the “outer” side of the double helix that is not in contact with the protein whereas the “inner” side closely resembles the conformation of a binding site without spacer (3KMD). (B) Different deformations of the inner and outer side of the double helix. The structures of the BAX RE (green) and 3KMD (magenta) are compared by a series of distances at the outer and inner side of the double helix, with respect to the protein-DNA interface. Only one distance at the outer side (red) varies significantly between both structures and indicates the compaction of the complex upon binding. (C) Distance measurements at inner and outer side of the DNA. The deformation of the BAX RE is most apparent for only one distance measurement at the outer side of the DNA (red).



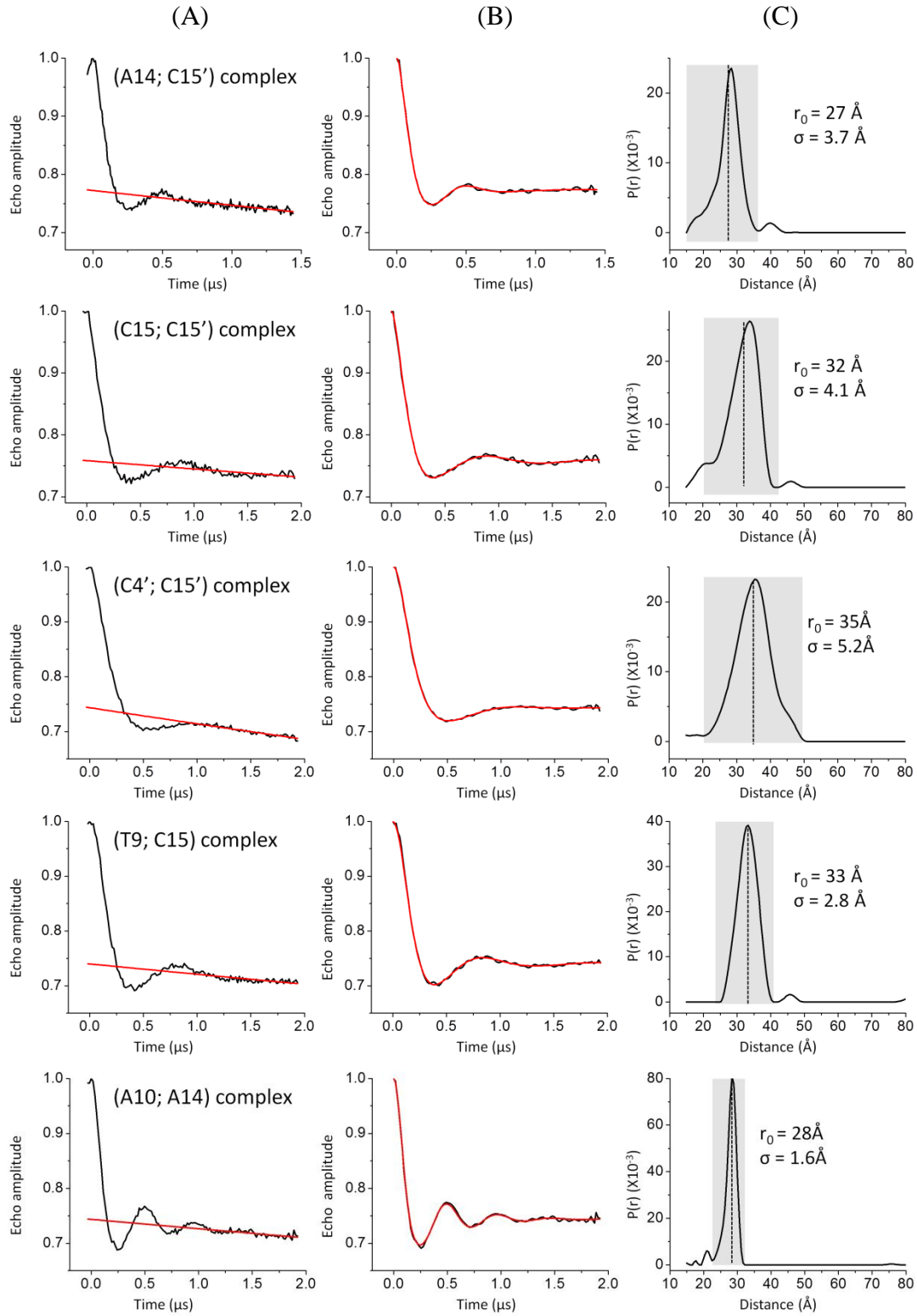
**Supplementary Figure S5:** Sequence of BAX-RE, with the two half sites identified by the blue rectangles and the nitroxide labeling sites circled in red. Inter-nitroxide distances measured in this study are indicated by the red lines. By convention, each phosphate ‘belongs’ to the ribose that is on the 3’-side of the P atom (1). Each distance set is denoted by the number and identity of the corresponding nucleotides (e.g., (A10; A14)).

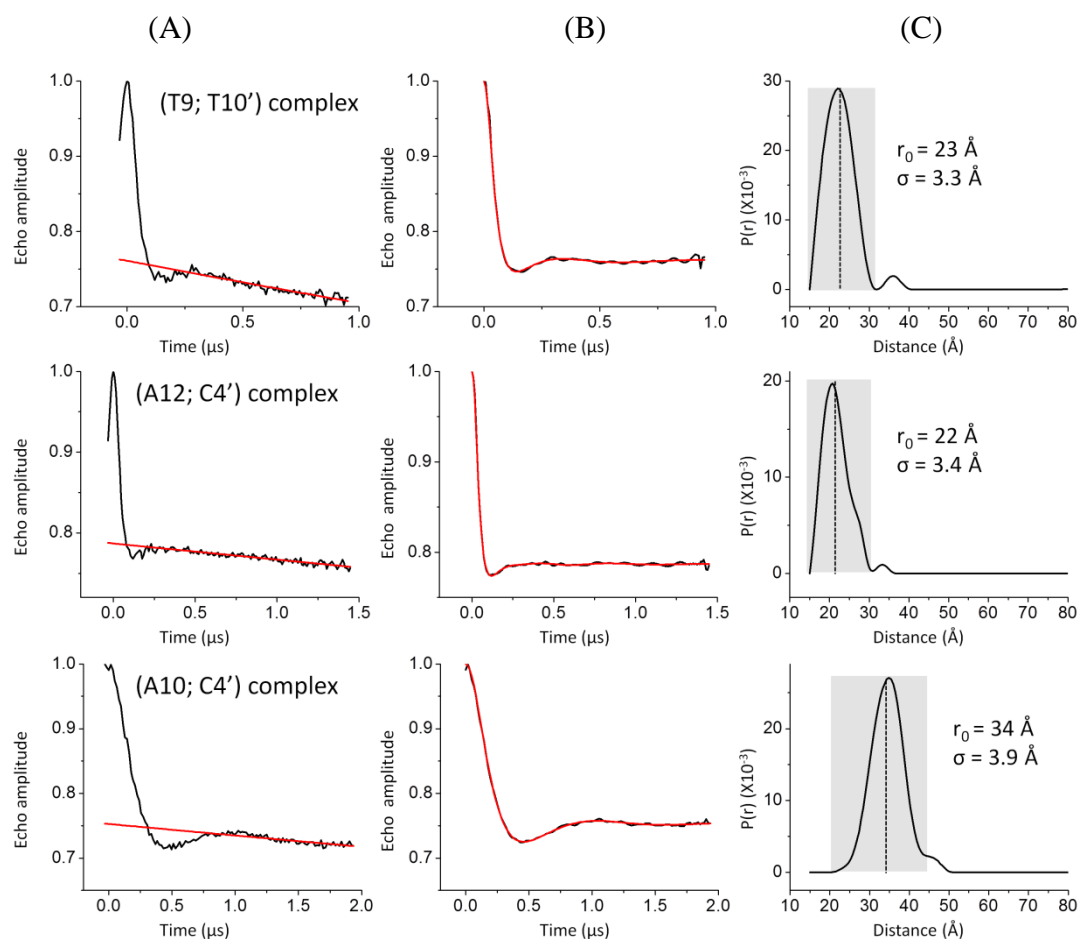




**Supplementary Figure S6: Example EMSA of samples used for pulsed EPR measurements.**

Samples 1-6 have a R5 pair labeled at (A14; C15'), (C15; C15'), (T9; C15), (A10; A14), (A10; C4'), and (A12; C4'), respectively. Each of the DNA-only samples (-) shows one band with the exception of sample 3 and 4, where 20% extra unlabeled F-chain was added to further ensure that all labeled E-chain was assembled into the duplex. Upon addition of p53 core domain (+), all DNA duplexes migrated as a retarded band, indicating that labeled DNAs were assembled into the DNA/p53-DBD complex.





**Supplementary Figure S7: DEER-measured inter-R5 distances in p53/BAX-RE complexes.**

Each data set is designated as described in the caption of Supplementary Figure S5A. (A) Original echo decay data. Shown in black is the measured echo decay that has been normalized to the amplitude at  $t=0$ . Shown in red is the dipolar decay background obtained by fitting the later portion of the data to an exponential decay corresponding to a homogeneous 3-dimensional distribution of electron spins (6). (B) Dipolar evolution functions. The black traces represent the differences between the measured echo decay and the background decay shown in (A). The red traces are the simulated echo decay computed according to the corresponding distance distributions shown in (C). Note that the dipolar evolution functions (black) were smoothed by applying a 1.6-nm long pass filter to remove the majority of the ESEEM effect (6). (C) Computed distance distributions  $P(r)$  (see Methods). Shaded boxes indicate the major bands in  $P(r)$  used to compute the mean distances ( $r_0$ , marked by the dotted line) and the standard deviations of the distribution ( $\sigma$ ) (1).

## SUPPLEMENTARY REFERENCES

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